JOURNAL OF DAIRY SCIENCE

SEPTEMBER, 1957

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Virus Resistant Mutants of Lactic Streptococci
Activities of the American Dairy Science Association

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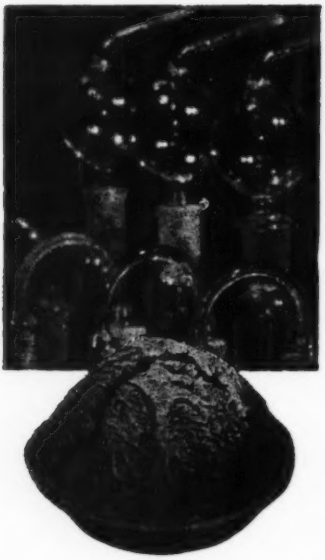

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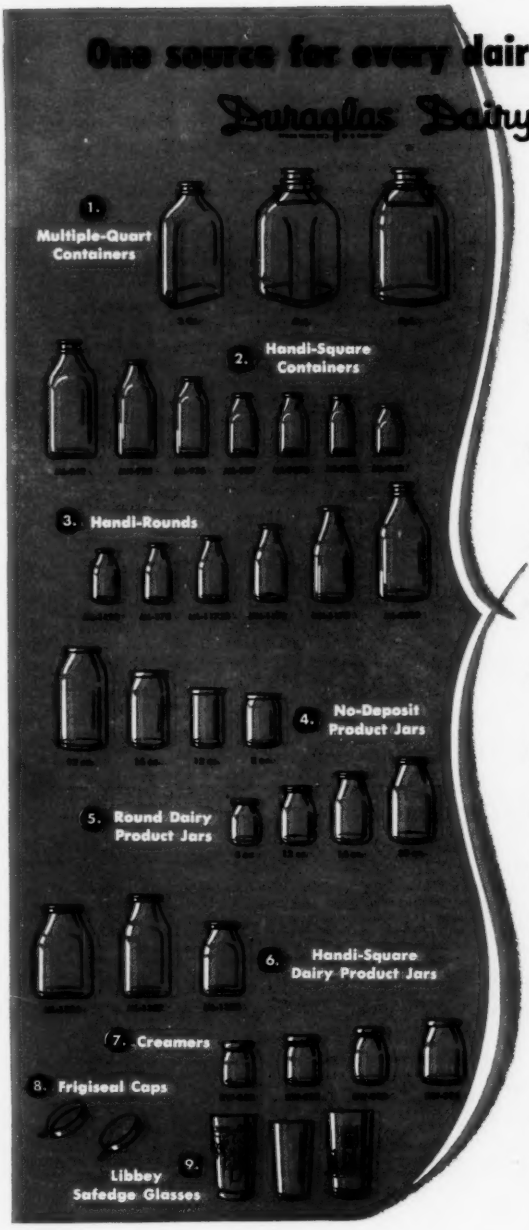

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PEOPLE and EVENTS
in the Dairy Science World

Pioneer Teacher, Researcher, and Author Retires

GUSTAV BOHSTEDT, distinguished teacher, researcher, and writer in the field of animal husbandry, will retire from his Univ. of Wisconsin teaching and administrative duties and then assume a nine-months' teaching assignment in September, 1957, as visiting professor in the Southern Illinois Univ. School of Agriculture, Carbondale.

Gustav Bohstedt

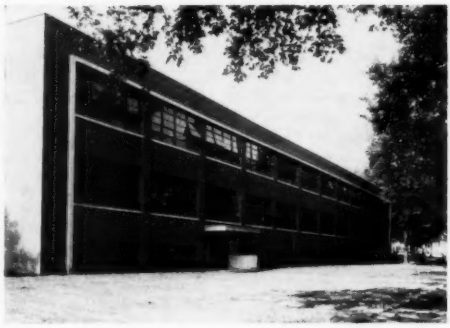
A native of Guissau, Germany, Dr. Bohstedt is retiring as head of the Univ. of Wisconsin Animal Husbandry Dept. He has been professor of animal husbandry there since 1928, and earlier taught there for five years (1915-16 and 1917-21). He earned his bachelor's, master's, and doctor's degrees from Wisconsin. He taught one year at Iowa State College (1916-17) and was chief of the Dept. of Animal Industry at the Ohio State Experiment Station for seven years (1921-28).

Dr. Bohstedt is a member of the American Dairy Science Association, the American Society of Animal Production, the American Association for the Advancement of Science, and a number of nonprofessional organizations. In addition to writing for various farm journals and periodicals, on the preservation and feeding qualities of hay and grass silage, he is the author or co-author of experimental station research bulletins and circulars dealing with animal nutrition and feeding studies. At Southern Illinois Univ., Dr. Bohstedt will teach in the Dept. of Animal Industry, a division of the School of Agriculture.

Mrs. Bohstedt, the former Ruth Conway, was born in Glendale, Montana. Before her marriage she taught school in Helena, and had among her sixth-grade students a boy named Frankie Cooper (son of Judge Cooper), who became known to the world as Gary Cooper. Mrs. Bohstedt's birthplace, Glendale, is now a ghost-town, but at one time it was a thriving mining community, with ambitions of becoming the capital of the Territory of Montana.

We welcome Dr. and Mrs. Bohstedt to the state of Illinois.

Plans for the Animal Disease Laboratory at Ames, Iowa



The Animal Disease Laboratory to be erected at Ames, Iowa, will provide expanded facilities for research on livestock diseases, and for disease diagnosis, control, and regulation. It represents another important step in the most accelerated attack on animal diseases that this nation has ever known.

Livestock, poultry, and their products account for more than half of the cash receipts in farm marketings. Production of red meat is about two-thirds higher than before World War II. Our people—170 million of them now—are eating, on the average, approximately 160 lb. of red meat per person a year.

These facts underscore the importance of keeping our livestock healthy and of stopping the economic waste caused by diseases and pests. Disease is the largest single handicap in livestock production. Together, diseases and pests cost livestock and poultry producers more than $2 billion a year. Mastitis, probably the most serious economic disease of cattle, costs about $225 million a year. Brucellosis accounts for losses of about $87 million, shipping fever about $25 million, and leptospirosis more than $110 million.

One step already taken in this accelerated attack on livestock diseases was completed with the establishment of a research laboratory on Plum Island, about two miles off-shore of the eastern tip of Long Island. Studies are under way there on animal diseases, such as foot-and-mouth disease, that exist in foreign countries and are a potential threat to this nation's livestock. (*Journal of Dairy Science*, December, 1956.)

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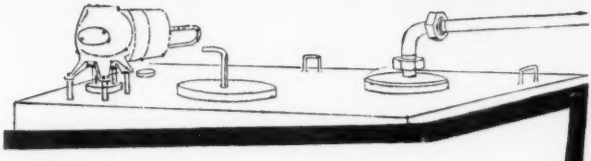

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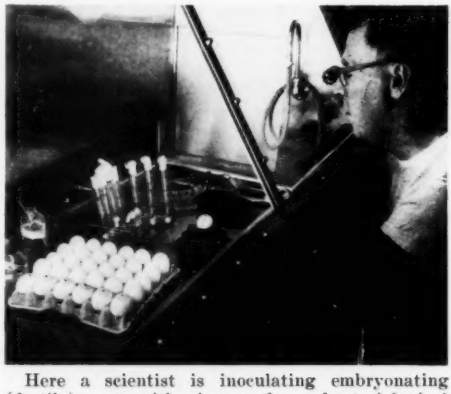
This step is the building of comparable facilities for research on livestock diseases occurring in this country. Funds for this purpose—$16½ million—were approved by the United States Congress, and a site has been selected near the Iowa State College, at Ames.

The architects and engineers currently are developing the design of the buildings, and expect to have final plans and specifications ready by the end of this year, with construction bids to be called for early in 1958. This would lead to expectation that the laboratory would be in operation in 1960.

Another step—expanded studies on animal parasites—will be taken as soon as the Animal Disease Laboratory is completed. When space now used for animal disease research at Beltsville, Maryland, is released, the parasitic disease studies can be expanded into the vacated facilities. Parasitic diseases, which make it impossible for animals to utilize feed satisfactorily, have a severe, though often unrecognized, economic impact on livestock production. It has been more than 20 years since any significant additions have been made to the Department's work with internal parasites of domestic animals. The laboratory at Ames indirectly makes possible this additional step in the expanded attack against animal diseases.

Farm area chosen for site. The new animal disease laboratory will be built on a 318-acre tract of farm land, which is two miles east and one-half mile north of Ames, Iowa. This site has been donated by the Iowa State College to the Federal Government for the Department of Agriculture. The College has offered use of its facilities, such as the extensive library of technical, scientific, and medical publications in the veterinary field, to members of the laboratory's staff. Also, its veterinary medical teachers and research staff will be available for consultation.



Here a scientist is inoculating embryonating (fertile) eggs with virus under a bacteriological hood. This illustrates one type of work which will be done in the new laboratory at Ames, and the kind of equipment used to prevent escape or cross-contamination of the disease.

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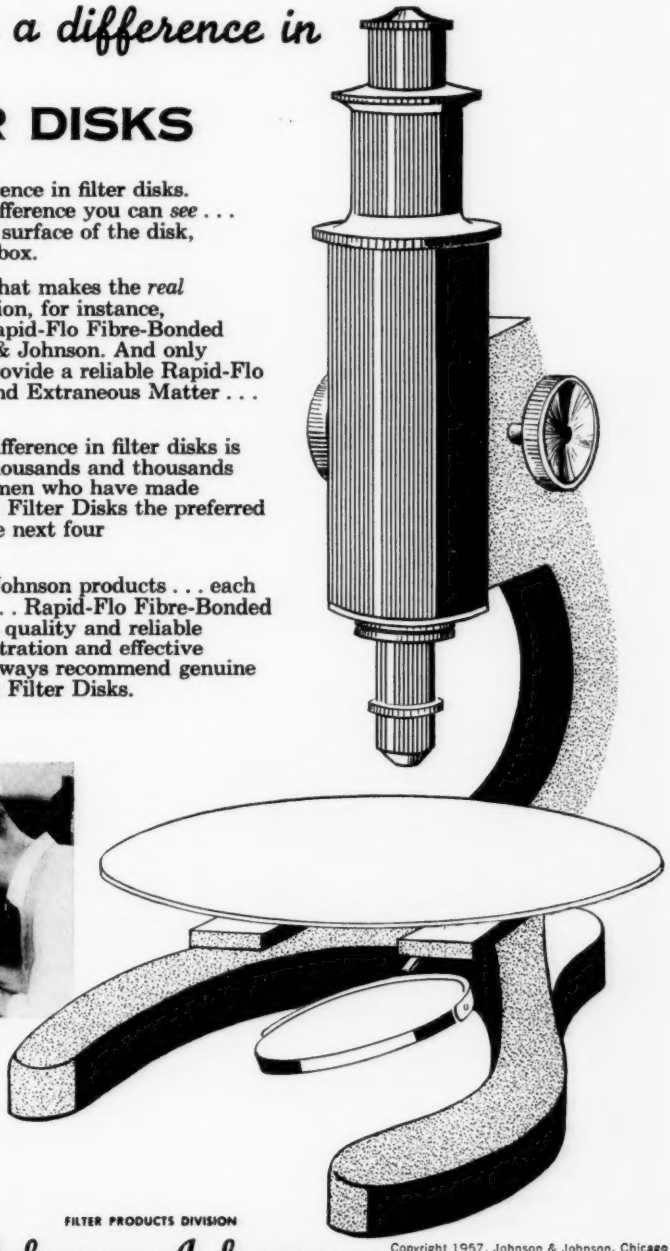

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The compound in which the main buildings will be enclosed will occupy about 30 acres of land. The remainder of the land will be used for quarantine, housing, and pasture for large and small animals to be used in diagnostic and research work. The compound will contain laboratory buildings, related service structures, and necessary office space.

The largest of the laboratory buildings is designed for research in which small animals such as guinea pigs, rats, mice, and hamsters will be used as test animals. They are cheaper to feed and easier to handle than the larger farm animals. Many of the fundamental studies can be made with small animals, although final checking of results must be made on the host animals—cattle, hogs, sheep, horses, or poultry.

As now planned, based on preliminary cost estimates, the small-animal laboratory will have 40 units for research and regulatory laboratory services, each unit completely isolated from the others. These will include units for related research in bacteriology, and other fields, as well as diagnostic work and biological products testing.

The five large-animal laboratories will be designed to permit two types of animal disease studies: initial-exploration and full-scale. In some cases, a statistically sound exploratory study can be made with as few as ten animals. A full-scale study requires many more, depending on the kind of experiment. Plans now specify ten large-animal units, each housing ten animals, for exploratory studies. There will also be ten units, each housing 40 animals, for full-scale research on disease in larger animals.

Model for safety. The new laboratory is expected to set standards for future construction of laboratories for the study of animal-disease problems. It will employ many new safety devices developed in biological research. These are to protect laboratory workers and the general public, to prevent escape of disease, and prevent cross-contamination of disease. Safety precautions will be similar to those in the foreign disease laboratory on Plum Island.

Air-locks will be installed at unit entrances. Pressure will cause air always to flow in, rather than out. The air-conditioning system will bring fresh air into each unit and exhaust it through heavy filters. Sewerage will be zoned to serve each unit separately. All sewage will be decontaminated.

Workers will enter the laboratory through dressing-rooms, where they will change from street clothes to laboratory uniforms. The change-rooms will be so arranged in the isolated units that employees will have to remove laboratory clothing and go through a shower to return to the dressing-rooms from the laboratories.

Maximum use will be made of devices such as biological hoods and Horsfall-Bauer units, which permit the handling of multiple disease-producing organisms without fear of cross-contamination.

Each area will be designed for versatility, so that it may be used either for research or

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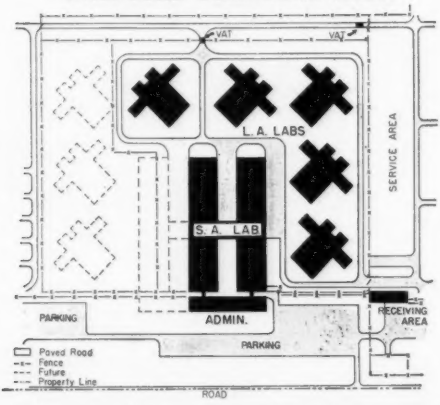
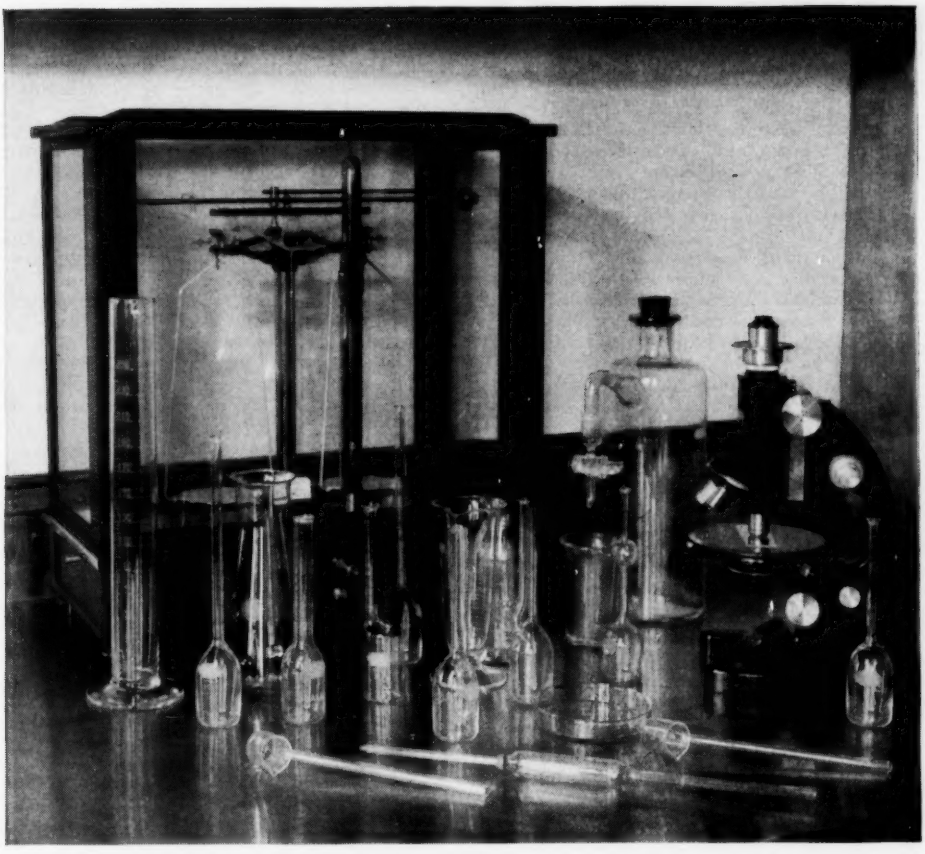


This preliminary drawing shows the proposed administration building, small-animal laboratory, five large-animal laboratories, and space for expansion. Plans for the 30-acre compound area are subject to change.



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for regulatory activities. About 80 per cent of the space will be allotted to research and 20 per cent to regulatory work. The regulatory activities will cover all aspects of the work—assistance in diagnosis, testing of samples from imported animals and products, testing of biologics, pilot scale trial production of biologics used in certain of the disease-eradication programs, and so on.

In addition to the compound and its immediately adjacent service structures, there will be barns and animal shelters, a sewage-decontamination plant, a steam plant, shops for the storage and maintenance of equipment, and storage buildings for feed. There will be quarantine and holding areas for incoming animals.

The laboratories will not be open to casual visitors after the work gets under way. People going into the laboratories will need passes, and will be required to observe the same rigid rules of dressing and showering that the employees follow.

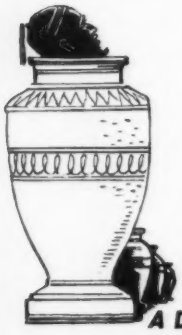
Broad scope of work. The addition of this laboratory to the Department's animal disease research facilities will provide the means for a considerably stepped-up research attack on domestic-animal diseases and parasites. In these more adequate facilities, scientists expect that as many as 500 large animals, 2,000 small animals, and 2,000 poultry may be used at one time in various experiments and laboratory tests. Under the present plan, it will be possible to conduct research on as many as 25 different animal diseases simultaneously.

Another index of the scope of the work is the wide range of scientific talent which will be required. About 100 principal scientists will direct and carry on the research and regulatory work—veterinarians, chemists, pathologists, physicists, and others.

In the never-ending war on animal diseases, it will be their responsibility to find new methods of bringing these enemies under control, and regulatory procedures to help put the findings into widespread use.

Diseases can strike at any time, anywhere. Sometimes we do not know where they come from. Sometimes, we do not even know if they are new, or if they are variants of existing diseases. Diseases in the mucosal complex seem to be new, with the first identified only about ten years ago. Vesicular exanthema is only a few years old, and so far has not been identified in any other country.

In spite of all efforts to keep them out, diseases can come in from other countries.



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There are 40 thieves lurking in every business operation today — awaiting every opportunity to rob it of profit. There are Wastes, Production Losses, Distribution Costs and a host of others — most of them well entrenched and not easily recognized.

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The Bureau uses a sharp and practised eye for ferreting out of Milk and Ice Cream operations the 40-odd leaks that may be robbing them of hard-won profit.

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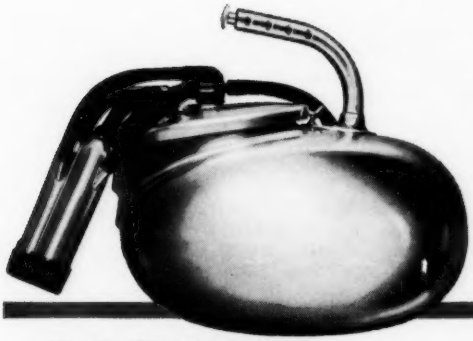

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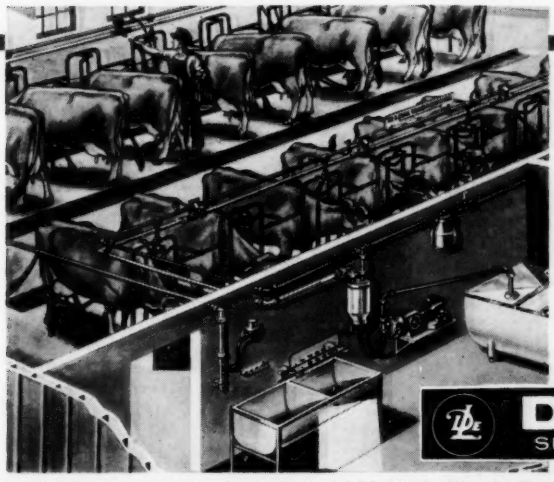

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Bluetongue, Newcastle disease, and the lancet fluke have all slipped past our quarantines within the past 20 or 25 years. These one-time foreign diseases have become established and must be studied along with other domestic diseases.

Modern techniques and transportation have served to spread diseases and pests farther and faster than was possible a few years ago. Research is needed to provide earlier detection of diseases, to keep pace with faster movements, and to suggest guidelines for preventing the spread of disease in commerce.

Other diseases, like mastitis, shipping fever, anaplasmosis, and leptospirosis, have been present for a long time and still are important problems for further research. With expanded facilities and intensified efforts, we can hope for a break-through to solve the remaining secrets of these presently perplexing problems.

Record shows major gains. We are getting results in many animal health problems. Many diseases once taken for granted, and their losses accepted as inevitable, are either being eliminated or brought under control. A hundred years ago, Texas fever in cattle was in this category. Then, a Department scientist discovered that the fever is spread by a tick. This led to control of the tick, as well as of insect vectors of many other serious livestock diseases.

The poultry industry as we know it today could not have developed without control of pullorum disease, coccidiosis, fowl pox, and other poultry diseases, made possible by research.

Fifty years ago it was not unknown to find dairy herds where 50 to 90% of the cattle were affected with tuberculosis. Research on methods of detecting tuberculosis and a widespread testing and elimination program gave us the key to bringing this disease under control. Today, the number of tuberculosis reactors in nine million cattle tested each year, is between one-tenth and two-tenths of 1%.

The combination of research and regulatory work has brought us to the point where we now dare to look ahead to eradication of brucellosis.

Research development of the milk ring test provided a quick and simple method for screening a herd for brucellosis. The blood agglutination test, developed earlier, pinpoints the infected animal or animals within the herd. Combining research findings with regulatory activities, the accelerated brucellosis eradication campaign was begun in 1954. Seven states are now classified as "modified certified free" of brucellosis. An additional 409 counties in 26 other states and Puerto Rico also have achieved this status. "Modified certified free" means that no more than 1% of the cattle and 5% of the herds of the total in the area may be infected with brucellosis.

Regulatory service outlined. Regulatory activities at the laboratory will divide into three

MULTI-PROCESS dual wall TANKS

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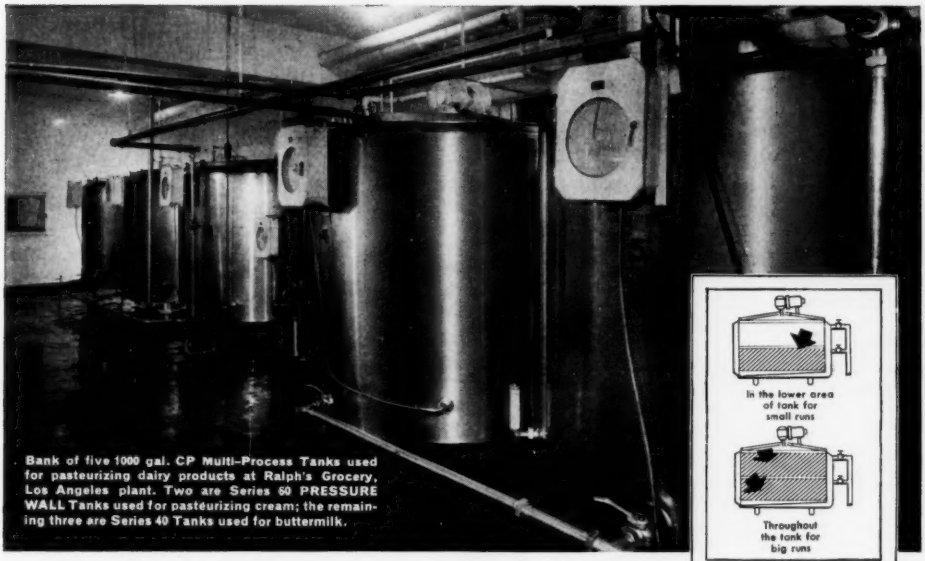

Bank of five 1000 gal. CP Multi-Process Tanks used for pasteurizing dairy products at Ralph's Grocery, Los Angeles plant. Two are Series 60 PRESSURE WALL Tanks used for pasteurizing cream; the remaining three are Series 40 Tanks used for buttermilk.

Puts the "Heat" on Dairy Processing . . . Chills, Too!

Out in California Ralph's Grocery is known for high quality standards. To maintain these standards Ralph's naturally selected CP stainless steel equipment for their dairy processing plant.

Their Multi-Process Tanks are primarily used for pasteurizing cream and buttermilk. However, due to the flexibility of the CP engineered equipment, they could just as easily switch from continuous batch operations to intermittent runs of other dairy by-products.

CP Multi-Process Tanks heat, pasteurize, mix, hold and cool speedily, efficiently and with a minimum of manual effort. Each installation can be tailored to suit your exact processing requirements. In capacities from 200 to 1000 gallons, CP Multi-Process Tanks are available for steam, vapor, hot water heating; or for chilled water or refrigerated cooling.

Whatever your processing needs, be assured you can rely on easy-to-clean, easy-to-maintain stainless steel CP Multi-Process Tanks. CP tanks give you more usable capacity with far fewer units . . . because each one can do so many jobs so well.

Talk your processing tank needs over with your CP Representative—or write for bulletins.

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Facilities of the Iowa State College library will be available to the staff of the Animal Disease Laboratory at Ames. The State Veterinary Diagnostic Laboratory on the campus at Iowa State College was completed last year. Its staff and that of the Veterinary Medical School will be available for consultation with the A.R.S. Animal Disease Laboratory staff.

main types of work—diagnosis, testing and control of veterinary biologics, and pilot-plant trial development of new biologics developed in the research program.

The laboratory will provide services in the diagnostic field which are not available elsewhere, and consultation service to the state animal disease diagnostic laboratories.

It will handle specimens of an unusual nature, sent in by the state diagnostic laboratories, or individuals, for testing and typing. The laboratory will also serve as a repository for pathological materials encountered in the field.

Future plans call for the organization of teams of trained diagnosticians from the laboratory, to be available for field investigations when diseases of unusual nature or high mortality or morbidity appear.

Testing of biologics has taken on increased importance with the expansion of the veterinary biological products industry. Doses of vaccines, bacterins, serums, and diagnostics totaled 2,307,000,000 last year—more than double the 1950 production. All indications are that this trend will continue.

This increase has been almost entirely in the production of "live" virus vaccines. Today more than four-fifths of the vaccines are of the live or modified live virus types.

Scientists in this field are faced with the challenge to develop the safest possible vaccines. At this laboratory, the Department will be setting standards for the determination of the potency, safety, and purity of all types of vaccines used in animals. Chemists will determine the active principles for dips and dis-

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If you are a public health official, you can take pride in the fact that fresh milk is now available almost everywhere in Canco disposable milk containers. Much credit for this advance goes to public health officials, who early recognized the disposable container as a great milestone on the road to better milk distribution.

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infectants used in animal disease control, and the preservatives used in biologies.

State work to continue. Two other aspects of animal health—the problem of contamination from industrial by-products and the effect on livestock health of the many different chemicals used in modern farming—will continue to be studied elsewhere. For instance, an effective spray to prevent certain fly infestations has been developed, but small amounts appear to be absorbed in the animal tissues. It needs further testing before release. The American people demand and have a right to expect that their food supply will be free from toxic materials. We need chemicals that will leave *no* residues harmful to man or animals. And we need research to give us that assurance.

That kind of research will be continued at the Agricultural Research Center at Beltsville, Maryland, or in cooperative projects with the states, and expanded as resources permit. The work at Ames will in no way supplant work being done in the states, for it will deal primarily with problems national in scope. Cooperative work with the states, dealing as it does with regional problems, is solidly established in our total research pattern. We expect to continue to receive major benefits from these joint efforts. The laboratory at Ames will support and strengthen the state cooperative work.

Completion of the Animal Disease Laboratory at Ames will begin a new era in the never-ending war on livestock diseases. However, completion of its modern buildings and facilities is not the end product. That is, to rid the nation's livestock of transmissible disease. Through this modern laboratory and the dedicated men and women who will staff it, we hope to come closer to that ultimate goal.

M. R. CLARKSON
Deputy Administrator
Agricultural Research Service, USDA
Washington, D.C.

American Butter Institute to Meet Oct. 11

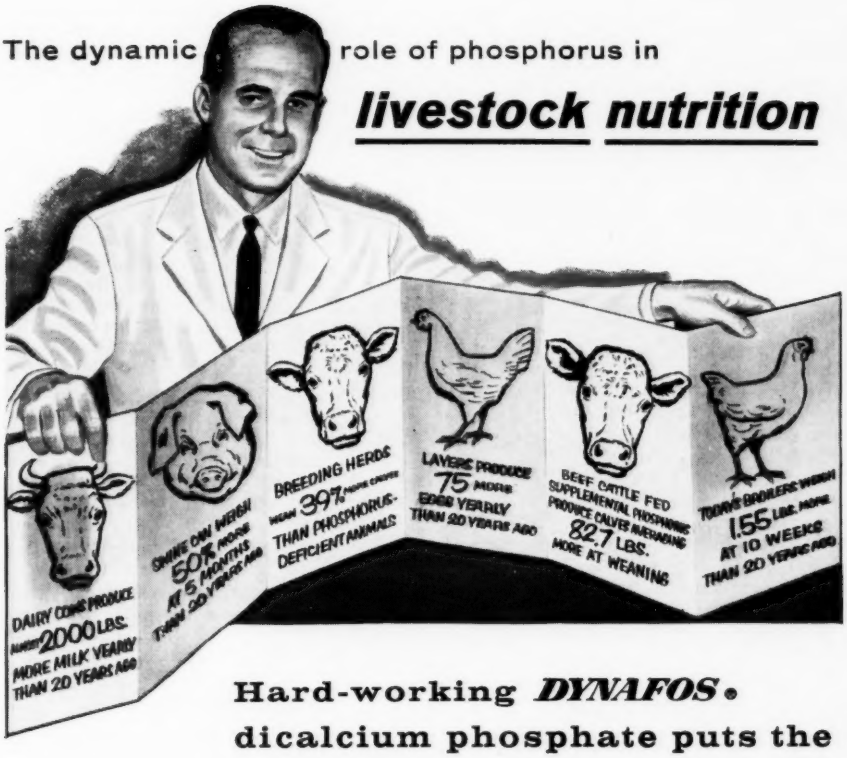
Program plans are rapidly being completed for the 49th Annual Meeting of ABI, which will be held October 11, at the Congress Hotel, Chicago.

Two outstanding national authorities who have already accepted invitations to address the ABI meeting are ROBERT SECREST, Commissioner of the Federal Trade Commission, and SHELBEY GREY, Director, Bureau of Program Planning and Appraisal, U. S. Food and Drug Administration.

Mr. Secrest is expected to explain accelerated efforts of FTC to insure truthful advertising in the presentation of competitive spreads and cooking fats. Mr. Grey usually acts as chairman or head of the Food and Drug officials, who meet at frequent intervals with the ABI-FDA Liaison Committee.

The dynamic role of phosphorus in

livestock nutrition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- Modern broilers need 79% more phosphorus than in 1932.

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University of Maryland News

R. D. APPLEMAN recently joined the staff of the Dairy Dept. as Extension Instructor. Mr. Appleman obtained his undergraduate and master's degree from Oklahoma State Univ., Stillwater. He recently completed a two-year tour of duty with the U. S. Army, in which he served in a research-with-livestock capacity at Edgewood Arsenal in Maryland.

R. G. SAACKE recently joined the staff of the Dairy Dept. as Instructor in Extension. Mr. Saacke obtained his undergraduate training at Rutgers Univ. and his master's degree in Dairy Cattle Breeding at Pennsylvania State Univ. He has recently completed a two-year tour of duty with the U. S. Army Signal Corps.

R. G. HEMKIN has joined the staff in the position of Ass't. Professor, with duties of teaching and research in Dairy Cattle Production. Dr. Hemkin obtained his Ph.D. degree from Cornell Univ., Ithaca, N. Y., last spring.

W. S. ARBUCKLE has returned to his position in the Dairy Dept. from a one-year sabbatical leave. During this past year, Dr. Arbuckle has had some association with the USDA Extension Service and has conducted a survey of teaching programs in dairy departments throughout the United States. On his return, Dr. Arbuckle will take the leadership in developing an extension program in dairy technology in the state of Maryland.

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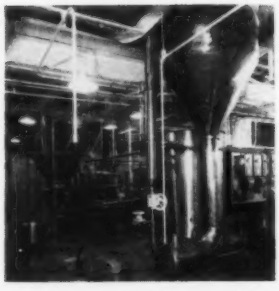

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Marvin L. Speck Honored by North Carolina

M. L. SPECK, Professor of Dairy Bacteriology, Dairy Manufacturing Section, Animal Industry Department, North Carolina State College, Raleigh, has recently been honored by being appointed to the William Neal Reynolds Distinguished Professorship in Dairy Bacteriology. The Distinguished Professorship is the highest honor bestowed upon professors in the School of Agriculture, North Carolina State College, who have distinguished themselves in research, scholarship, and performance.

The Distinguished Professorships are made possible by an endowment contributed by the late WILLIAM NEAL REYNOLDS of the R. J. Reynolds Tobacco Company. Dr. Speck is a member of the Editorial Board of the *Journal of Dairy Science*.

Members of the Animal Industry Dept. staff who have previously been named recipients of the William Neal Reynolds Distinguished Professorships are GEORGE H. WISE, Head, Animal Nutrition Section, and J. E. LEGATES, Head, Dairy Husbandry Section.

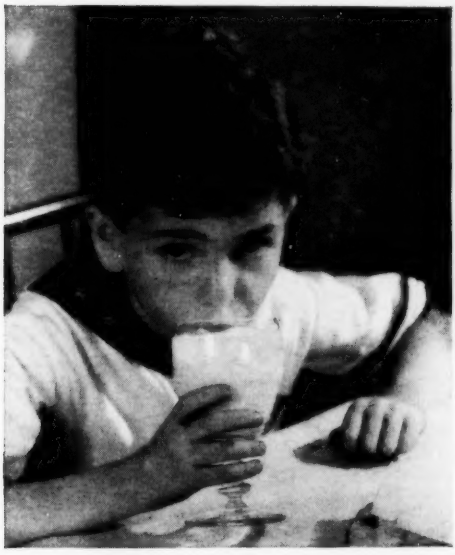
North Carolina News

Four Dairy Manufacturing students at North Carolina State College have been awarded scholarships for the 1957-58 school year. JAMES RUFUS BATTEN, Middlesex, and ATLAS GORDON NEVILLE, Chapel Hill, were awarded a $500 one-year scholarship for freshmen in Dairy Manufacturing. These scholarships were made available by contributions from the North Carolina Dairy Products Association.

JOSEPH RAY CARLYLE, Kinston, was awarded a $300 one-year scholarship for a sophomore in Dairy Manufacturing. This scholarship also was made available by contributions from the North Carolina Dairy Products Association. Mr. Carlyle was also one of the recipients of a North Carolina Dairy Products Association scholarship last year, given for freshmen in Dairy Manufacturing.

JAMES DARRELL PUNCH, Conover, is the current recipient of a $500-a-year scholarship. This scholarship for students majoring in Dairy Manufacturing or Dairy Husbandry is sponsored by the Tarheel Supplymen's Association. Mr. Punch was the recipient of this scholarship last year also.

The American Dairy Association of North Carolina has recently established a college scholarship program. The scholarship is for a worthy



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girl or boy to use to obtain a college education. The annual scholarship of $300 is to be alternated between a boy and a girl each year, for the freshman year in college. The boy's scholarship is for a college in North Carolina; for the girl, it is for the study of Home Economics at an approved college in North Carolina. The recipient must have as a parent a member of the American Dairy Association of North Carolina, which has over 11,000 members.

The American Dairy Association of North Carolina Scholarship will be awarded on the basis of: *(1)* scholarship; *(2)* leadership; *(3)* interest, and *(4)* need.

Completed Theses

M.S. Degree

DILL, CHARLES WILLIAM—The interrelationships of heat treatment, solids-not-fat, and calcium chloride to the yield of cottage cheese. North Carolina State College, Raleigh.

YOUNG, JOSEPH EARL—The relationships of types and concentrations of stabilizers, concentrations of cocoa powders, methods of processing and days in storage, to viscosity and sedimentation in chocolate milk. North Carolina State College, Raleigh.

Five Boys Win $1,000 Dairy Scholarships

Winners of five dairy technology scholarships to the Univ. of Illinois—each worth $1,000 over four years—have been announced by P. H. TRACY, head of dairy technology work at Illinois.

These scholarships were provided by dairy plants in the state to recruit and train qualified people for the dairy manufacturing industry. Winners and donors this year are: BENNY COPLAN, Kewanee, sponsored by Illini Dairy Queen, Inc., Springfield; DALE DORNBLASER, Hume, sponsored by Bowman Dairy Company, Chicago; LOUIE D. KING, Pulaski, sponsored by Johnson & Johnson, Chicago; CHARLES E. KOEHLER, Rock Falls, sponsored by Pure Milk Association, Chicago, and CLIFFORD R. THOMAS, Erie, sponsored by Brook Hill Farms, Chicago.

These boys were selected on the basis of scholarship in high school, from among a number of applicants. More of the same type of scholarships are yet to be awarded.

Carnation Promotes Lindsey

J. B. LINDSEY, Chicago district sales manager for grocery products for the Carnation Company, has been named merchandising manager of Carnation Instant Products Division, Los Angeles.

A graduate of Stanford School of Business, Lindsey joined the Carnation Company seven years ago under the Company's college recruitment program. He served on the San Francisco sales force, was supervisor of the Oakland, Calif. office, and district sales manager in Milwaukee, Wisconsin, before taking over his Chicago post. He assumed his new duties on August 15.

Indianapolis District Manager R. L. LOGSDEN was named to succeed Lindsey in the Chicago office. A. C. BECKS, assistant district sales manager, St. Louis, was appointed to the Indianapolis district managership.

Ohio State News

A new type of short course in the Department of Dairy Technology has been developed. A five-months' correspondence course in Market-Milk will be offered between October 14, 1957, and March 14, 1958.

The course work generally will be handled by weekly assignments, to be completed at home and on the job. In addition, five one-day periods have been scheduled at the University during the course, for laboratory work, discussions, and examinations. Registration cost for the course will be $50.

The new course, which is being offered following a survey of dairy leaders in Ohio, will replace, for this year, the regular two-weeks' short course in Market-Milk. The objectives of the course are to teach proper methods of product handling, sanitation and public health aspects, and application of elementary chemistry and bacteriology, as they pertain to the market-milk industry.

Other courses offered in the Adult Education program of the Department of Dairy Technology includes: Short Course for Ice Cream Makers, January 6-17, 1958; Dairy Technology Conference (Silver Jubilee Year), February 25-28, 1958, and Short Course in Sanitation and Public Health, March 17-21, 1958.

Thirteen sustaining and 15 one-year, subject-to-continuation type scholarships have been awarded to 14 freshmen, ten sophomores, two juniors, and two seniors in the Department of Dairy Technology for the coming year. The scholarships amount to $7,700. The donors include Roy W. Babcock, Toledo; Beatrice Foods Co., New Bremen (2); Broughton's Farm Dairy, Marietta; Central Ohio Dairy Technology Society; Cincinnati Bottle Exchange; Columbus Milk Distributors Association (4); Dayton Milk Foundation (3); Esmond Dairy, Sandusky; Hopewell Dairy, Bellefontaine; Maumee Valley Dairy Technology Society; Meyer Dairy Products Co., Cleveland; Northeastern Ohio Dairy Technology Society (4); Sealtest Dairy Co., Cleveland (2); S. L. Sauffer, Warren (4), and the Robert B. Stoltz Memorial Fund.

K. M. SHAHANI, Research Associate in the Dept. of Dairy Technology for the past four

years, has accepted a position as Associate Professor in the Dept. of Dairy Husbandry, Univ. of Nebraska, effective September 1.

D. A. SEIBERLING has resigned his position as Instructor in Dairy Technology, to go into commercial work.

New Booklet Details Judging and Scoring Milk

Issued this month by the USDA is Farmers' Bulletin No. 2111, which describes some of the fundamentals of judging and scoring milk.

It tells how to detect the various odors and flavors, and how to prepare samples. It describes the score-card method of judging milk, and how samples are rated for odor and flavor, sediment, container and closure, bacteria, and temperature.

Irish Dairy Chemist Visits Wisconsin

G. T. PYNE, Professor of Dairy Chemistry, Univ. College, Cork, Ireland, recently paid a visit to the United States, where he visited several university dairy departments and dairy research laboratories.

While at the Univ. of Wisconsin, Madison, on July 23, he addressed a group of staff members and graduate students from the Departments of Dairy and Food Industries and Biochemistry. Dr. Pyne discussed his research on factors affecting the coagulation of milk.

Cornell News

The Dept. of Dairy Industry, Cornell Univ., Ithaca, N. Y., held a training school for farm bulk-tank truck drivers on Wednesday afternoon and evening, Aug. 21.

In spite of the truck-drivers' strike, 24 men took part in the program.

Notices were sent out, with the aid of county agents, only to the counties adjoining Tompkins County, because this training school will be offered at various strategic points around the state this winter.

Representatives were from the Dairymen's League, Sheffield Farms, Fiorlst of Watkins Glen, Marble Farms of Syracuse, Newark Milk & Cream of Owego, Four-County Creamery of Harford, Syracuse Dept. of Health, N. Y. State Dept. of Health, N. Y. State Dept. of Agriculture and Markets, and a number of independent haulers.

DR. WHITE spoke on basic sanitation, composition of milk, fundamentals of bacteriology, and judging of milk quality. PROF. MARCH discussed the proper procedure for sampling, measuring, and picking up of milk on the farm by tank truck drivers.

To date there are 2,828 bulk tanks on farms in New York State and 250 bulk pick-up routes.

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Fourth Edition

DAIRY BACTERIOLOGY

By B. W. HAMMER, Dairy Consultant;
and F. J. BABEL, Professor of Dairy Bacteriology, Purdue University,
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PREPARATION OF A CASEINATE MODEL SYSTEM[1]

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A fundamental basis is contributed for studying physical and chemical changes resulting from processing and storing of concentrated milk. A simple model caseinate system was prepared by supercentrifuging the calcium caseinate–calcium phosphate complex from skimmilk, either fluid or reconstituted from low-heat nonfat dry milk, and then dispersing the complex in air-free whey, water, or artificial sera at 4° or 60° C. A "residual fraction" of five to ten per cent of the original casein remained in the supernatant. Redispersing caseinate in water formed a new residual fraction, but in artificial sera containing citrate, sodium, and calcium, more of the residual fraction developed, indicating dissociation of the complex and sodium caseinate formation. Model systems of the concentrated caseinate increased in viscosity during storage, and gelled. *Editor.*

Calcium caseinate–calcium phosphate complex is the major colloidal component of milk, and undoubtedly the most critical one in the changes resulting from processing and storage of milk products. To elucidate these changes it is logical, therefore, to start with the preparation of a caseinate model system and to study the colloidal structure of the system, as affected by concentration, aging, heating, addition of electrolytes, whey constituents, and other factors.

This study deals mainly with the preparation of a caseinate model system, by isolation and redispersion of the calcium caseinate–calcium phosphate complex to a state comparable to its original colloidal state in milk.

EXPERIMENTAL PROCEDURE

Isolation of caseinate from skimmilk. Sedimentation in a supercentrifuge was used to obtain caseinate with composition and properties as near as possible to those of the native caseinate in milk. Skimmilk (heated at 63° C. for one-half hour) was obtained from the University Creamery. For some experiments, where a uniform source of caseinate was desirable, the skimmilk was made up from a low-heat nonfat dry milk[3] by dissolving it in ten times its weight of distilled water. The reconstituted milk was passed through a 100-mesh screen and held at 4° C. overnight before use. Sedimentation of small quantities of skimmilk (40 to 320 ml.) was accomplished in a Servall Superspeed Angle Centrifuge, and larger quantities (two liters) in a Sharples Supercentrifuge. From preliminary runs, it was established that centrifuging for from two to three hours at 14,000

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[2] Present address: Eastern Utilization Research Branch, USDA, Washington, D.C.

[3] Obtained from Challenge Butter and Creamery Association; examination indicated very little or no denaturation of whey proteins.

r.p.m. in the Servall (temperature about 40° C.), or for ten minutes at 45,000 r.p.m. in the Sharples (temperature about 30° C.), decreased the casein content in supernatant liquid to a limit. A residual fraction of from five to ten per cent of the original casein could not be removed; this finding is in agreement with results of Ford *et al. (3)*. Skimmilk was passed through the Sharples Supercentrifuge (clarifier bowl) at a flow rate of about 1,200 gm. per hour, to separate the caseinate.

The wet caseinate sediment was used as such, or after conversion into a dry state by freeze drying at 0.1-mm. pressure. The freeze-drying technique was applied in two different ways: *(1)* the wet sediment was transferred into conventional lyophilizer flasks, frozen overnight at −15° C., and then lyophilized; and *(2)* the caseinate deposited in the Sharples clarifier bowl was used without transfer. In the latter case, the bowl was taken from the centrifuge and placed horizontally in a freeze-drying apparatus; the inlet opening was connected with the low-temperature trap, and the discharge openings were connected with a manometer (shown in Figure 1). Powdered dry-ice was placed around the bowl

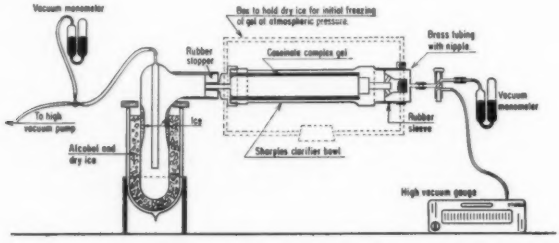

FIG. 1. Apparatus for freeze drying of caseinate complex gel in Sharples clarifier bowl.

for one hour, to freeze the sediment. After starting the vacuum and removing the dry-ice, the bowl was wrapped in a one-cm. layer of cotton for insulation, to prevent melting of the frozen sediment. About 24 hours were required to complete the lyophilizing, in both procedures. The dry material was powdered in a mortar and passed through a 100-mesh screen. Finally, it was placed in a vacuum, over P_2O_5 for three days.

Redispersion of the caseinate. Dispersion of the sediment to a colloidal state like that in skimmilk was found very difficult. Both the Waring Blendor and the Servall Omnimixer produced incomplete break-up, with troublesome foam formation. Foaming was prevented by dispersing in vacuum with a magnetic stirrer or in a rotating flask, using glass beads, in both cases, to break up the particles. The rotating-flask procedure, improved by use of a large quantity of glass beads, was finally adopted. The "initial" dispersion (150 to 200 ml. with casein content similar to that of skimmilk) and glass beads (four-mm. diameter, 200-ml. bulk volume) are placed in a stoppered bottle (500-ml. volume) with a stopcock. After evacuating to about two-cm. pressure, the bottle is placed with the bottom half in water of 50 to 60° C. (stopcock closed) for 15 minutes. This removes dissolved air by boiling the liquid. Repeated redrawing of the vacuum

results in complete removal of air from the bottle. A perfect vacuum with only water-vapor present is essential to prevent foaming, which causes insufficient dispersing action of the beads. The bottle is rotated end-over-end (100 to 120 r.p.m.) at 4° C. for 24 hours, or at 60° C. for one to two hours. Both temperatures are suitable to inhibit the growth of organisms. The low temperature was chosen for this study. Large quantities were handled in two-liter bottles (700-ml. dispersion, 850 ml. beads per bottle) rotated at 100 r.p.m. The initial dispersion of wet caseinate gel was obtained by pressing it through a fine-mesh stainless steel kitchen strainer with the dispersion fluid. Dry caseinate was mixed with beads in the bottle, and the dispersion fluid was added after evacuation, followed by immediate rotation for 15 minutes to obtain the initial dispersion. Dispersion fluids were whey from the supercentrifuge, distilled water, or "artificial sera." Artificial Sera I and II were prepared with 0.09 g. of calcium [weighed as calcium citrate $Ca_3(C_6H_5O_7)_2 \cdot 4H_2O$], 2.00 and 0.70 g. of citric acid, 1.07 and 0.34 g. of sodium hydroxide, per liter, to pH 7.0 and 6.6, respectively. Final dispersions were concentrated, if desired, at 28-mm. pressure (b.p. 28° C., water-bath temperature below 60° C.). The degree of concentration was controlled by weighing the concentrate and original liquid.

Examination of the state of dispersion. A routine test, based on the rate of sedimentation in a Servall Superspeed Angle Centrifuge at 14,000 r.p.m., was developed. Stainless steel tubes centrifuged for different periods were placed in a kathetometer type of apparatus (tube-holder instead of telescope). A small-diameter glass tubing with a U-bend at the end was placed in a fixed position above the liquid and connected with sampling tubes to a suction line. Samples were collected by moving the centrifuge tubes up a measured distance. The first three millimeters of liquid, usually containing some butterfat, was discarded. In the tests with 40 ml. skimmilk or dispersion of similar casein content, centrifuged for one-fourth hour, three fractions were collected between 3 to 20, 20 to 37, and 37 to 54 mm. distance from the top of the supernatant liquid (average distances were 11.5, 28.5, 45.5 mm., respectively). A layer 16 mm. above the bottom with gel and "fluid sediment" was left in the tubes. When centrifuging concentrates, 45-ml. samples were used because of the larger gel and fluid sediment, and therefore a 25-mm. layer was left at the bottom. Each fraction was tested for nitrogen by Kjeldahl determination.

Results expressed as per cent (B) of the original casein were calculated as follows: Total nitrogen in the supernatant fractions was first expressed as percentage (A) of total nitrogen in the original sample. Conversion into the desired percentage (B) was then $B=\frac{100\ (A\text{-}C)}{100\text{-}C}$, where ($C$) is the total nitrogen in the caseinate-free supernatant whey expressed as percentage of the original total nitrogen. $C=\frac{100\ D}{100\text{-}E}$, where ($D$) is noncasein nitrogen in the original sample expressed as percentage of the original total nitrogen; (E) is a correction factor for the removed caseinate. $E = 6.89 \times 1.52 \times F$, where ($F$) is the percentage of casein nitrogen in the original sample. The conversion factor for casein nitrogen to dry caseinate colloid (free of whey) is 6.89; the conversion

factor for hydration of the colloid, 1.52 (*3*). In the calculation it is assumed that only casein nitrogen is removed.

This method of nitrogen depletion can be duplicated easily. Storage of skimmilk at 4° C. for 24 hours, or holding the centrifuged tubes at room temperature for one hour before taking the fractions, was not critical. Treatment of skimmilk in a Raytheon sonic oscillator, at 4° C. with ten kilocycles for one hour, produced much higher nitrogen content in the supernatant, indicating break-up of the caseinate particles in skimmilk.

The state of dispersion was given a preliminary examination by measuring the sediment of a ten-ml. sample after centrifuging at 2,500 r.p.m. for one-half hour (International Centrifuge). This test is suitable to follow the disintegration of large particles, after various time intervals during dispersion procedures.

Analysis. A semi-micro digestion and distillation procedure was used for the total nitrogen, and the Rowland (*4*) procedure for noncasein nitrogen and nonprotein nitrogen. Viscosity determinations were made with the Fisher Electroviscometer at 25° C. The apparent viscosity was measured after various periods during a 15-minute run. Structural viscosity is then shown by decreasing values, as a result of stirring the liquid with the bobbin in the viscometer.

RESULTS

The isolated caseinate contained from 35.3 to 37.3% total solids (calculated from total solids contents of dispersions, from the loss in weight by freeze-drying, or from the difference in total solids of skimmilk and whey from supercentrifuging). This indicates that the original voluminosity of the caseinate, as given by Eilers (*2*) (about 200% of water), was maintained in the gel.

Dry caseinate was easy to disperse, could be stored without change, and was useful as a supply of uniform caseinate for experimentation. The powdered dry material contained about six per cent moisture. When the dry caseinate with six per cent moisture was exposed to the atmosphere in a 1-mm.–thick layer, the moisture content increased to ten per cent in one hour and to 12% in six hours, indicating that it is extremely hygroscopic. Final drying in vacuum over P_2O_5, in layers about 1 cm. thick, reduced the moisture content to 0.6% in three days, and to zero in three weeks. When the layer of caseinate was cut down to about 1 mm. in thickness, the moisture was reduced to 0.3% in three days and to zero in two weeks. Prevention of shrinkage during freeze-drying is important, to retain the original dispersibility. Transfer of the wet gel to lyophilizing flasks and freezing overnight or to too high a local temperature during freeze-drying, may cause part of the caseinate to dry in the unfrozen state, with resultant shrinkage and the formation of much stronger bonds connecting the particles. This material was very tough, like regular dry casein, and insoluble in water; whereas, material dried in the frozen state was brittle and easy to disperse.

The collodial state of caseinate dispersions in comparison with skimmilk is shown (Figure 2). Dispersions of wet caseinate or shrinkage-free dry caseinate

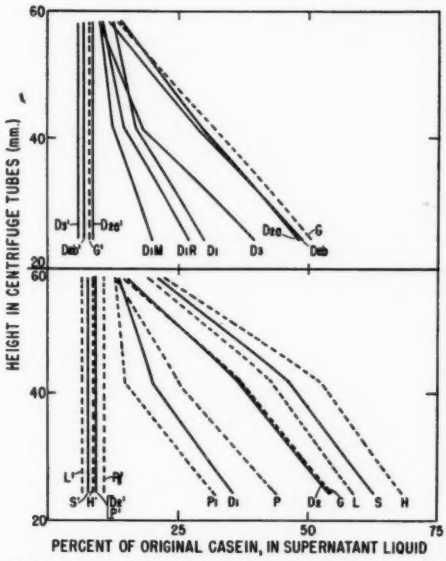

FIG. 2. Colloidal state of caseinate dispersions and skimmilk. Upper graph = caseinate in water. Lower graph = caseinate in whey. Sloping lines = one-fourth hour Servall. Vertical lines = three hours Servall (prime series). S = skimmilk, October–November, 1955, average. H = skimmilk, maximum values. L = skimmilk, minimum values. G = wet caseinate gel. D_1 = dry caseinate. D_1R = dry caseinate, dispersed with small quantity of beads. D_1M = dry caseinate, dispersed with magnetic stirrer. D_2 = dry caseinate, shrinkage-free (a and b = duplicates). D_3 = dry caseinate, shrinkage-free, coarse fraction. P = reconstiuted nonfat dry milk, low-heat. P_1 = reconstituted nonfat dry milk, heated in the concentrated milk stage.

were similar, but not quite the same as skimmilk. Freeze-drying without special precautions produced less dispersible dry caseinate (D_1) because of shrinkage, with dispersion properties similar to those of two experimental skimmilk powders (P and P_1). D_1M, D_1R, and D_1 show development to the final method of redispersion. Caseinate D_3 was obtained by isolating a coarser fraction (65% of the total casein) at a lower speed (43,000 r.p.m.) and higher flow rate (1,500 gm. per hour), in the Sharples Supercentrifuge. Dispersion in water, as compared to whey, resulted in somewhat greater sedimentation (less casein in the supernatant liquid) under the same conditions, probably due to the lower viscosity and specific gravity of the medium. Sedimentation from both media after prolonged centrifuging was to similar limits, which indicates that a residual fraction is formed anew upon dispersion of the isolated caseinate in water. The variation (L-H) in skimmilk from the October–November, 1955, period may be caused by differences in the fluid milk or in the processing. To determine the effect of the experimental treatment, it is desirable to compare the treated sample with a control of the same untreated milk.

Further experiments were carried out with caseinate from a uniform source: low-heat nonfat dry milk (Methods section). Reconstituted skimmilk from this

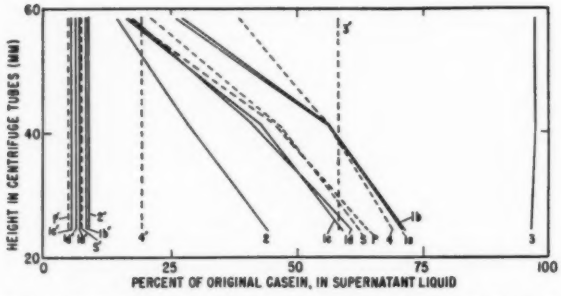

FIG. 3. Effect of dispersing temperature and medium on the colloidal state of caseinate. Sloping lines = one-fourth hour Servall. Vertical lines = three hours Servall (prime series). S = fluid skimmilk. P = reconstituted skimmilk from low-heat nonfat dry milk. 1 = caseinate gel dispersed in whey: a = 18 hr. at 4° C., b = 42 hr. at 4° C., c = one hour at 60° C., d = two hours at 60° C. 2 = caseinate gel dispersed in distilled water. 3 = caseinate gel dispersed in artificial Serum I. 4 = caseinate gel dispersed in artificial Serum II.

source was similar in colloidal state to fluid skimmilk (P and S, *see* Figure 3). Dispersion of the caseinate was much faster at 60° C. than at 4° C.; however, the ultimate limit of dispersibility appeared somewhat higher at 4° C. (1 a-d, *see* Figure 3). Curves 2, 3, and 4 demonstrate the effect of the medium. The rate of sedimentation in the one-fourth hour test was greater when the caseinate gel was dispersed in water, less when dispersed in Serum I, and about the same when dispersed in Serum II, as compared with that of a dispersion in whey (*see* Figure 3: 1a, 2, 3, and 4). The dispersion in Serum I was only slightly milky, suggesting the predominance of sodium caseinate, which does not sediment in the Servall Superspeed Angle Centrifuge. The high concentration of sodium citrate and the relatively high pH of 7.57 of the suspension in Serum I can account easily for the formation of sodium caseinate. The high residual fraction of the caseinate dispersion in Serum II, with less sodium content and a normal pH of 6.71, indicates the presence of some sodium caseinate (*see* Figure 3: 4′).

Skimmilk and caseinate dispersions in water and sera (P, 2, 3, 4, *see* Figure 3) were concentrated. The rate of sedimentation of the concentrates (*see* Figure 4) was much greater from water and Serum II (low viscosity and specific gravity of the medium), closer in range but different in characteristics from Serum I, as compared to skimmilk concentrate (*see* Figure 4: 3 and P, different slopes). Storage produced viscosity increase due to structural viscosity and decrease in sedimentation. Concentrate 3 formed a gel that thinned upon transfer and exposure to higher temperature. The viscosity measurements are recorded (Table 1). Concentrates 2 and 4 were milky, Concentrate 3 was slightly milky and similar in appearance to the original dispersion; pH values of the stored concentrates were 6.9, 6.7, and 7.25, respectively. Heating Concentrate 3 at 85° C. for one-half hour produced a distinct milkiness (*see* Figure 5) with decrease in viscosity and pH (7.13 after heating) and increase in sedimentation (3H, *see* Figure 4), with perfectly clear supernatant fractions. The formation of milkiness was reversible; it gradually disappeared in storage but no gel was formed within

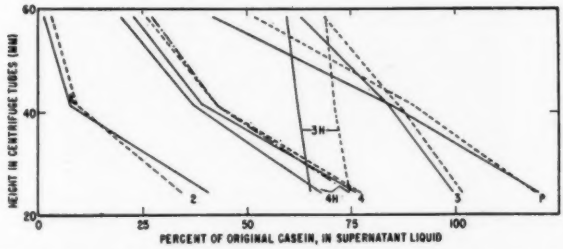

FIG. 4. Sedimentation of caseinate from concentrates. Three hours Servall. Drawn lines = fresh concentrates. Dotted lines = after storage at 4° C. for one day.

P = reconstituted skimmilk from low-heat nonfat dry milk. 2 = caseinate gel + water. 3 = caseinate gel + Serum I. 3H = 3, heated one-half hr. to 85° C. 4 = caseinate gel + Serum II. 4H = 4, heated one-half hr. to 85° C.

three days at 4° C. Sedimentation of 3H decreased in storage, with reappearance of turbid supernatant fractions, but remained different from 3 in other characteristics (different slopes of 3 and 3H, *see* Figure 4). Concentrate 4, when heated, showed changes in the same direction as 3, in a slight degree. No change in milkiness was observed in 4. The increase in milkiness of Concentrate 3, when it was heated, indicates the formation of calcium caseinate aggregates, which gradually revert to the original equilibrium of sodium caseinate and calcium caseinate upon further storage at 4° C.

Results with artificial sera indicate a substantial change from calcium caseinate complex to sodium caseinate under influence of the electrolytes. The calcium level (0.09 gm. per liter) chosen for the sera was that given by Van Kreveld and Van Minnen *(5)* as being present in milk in ionic form. The total calcium content of these sera would be very much lower than that of milk sera, which have another 0.33 gm. of calcium per liter in undissociated form. In Serum I, the citric acid level was the same as in milk; according to Clark *(1)* the alkali level considerably lower (in milk about 2.6 gm. per liter expressed as NaOH). In Serum II, the amount of sodium citrate was the minimum necessary to dissolve the calcium.

TABLE 1

Viscosity measurements of various concentrates

Nature of concentrates	Casein	Viscosity at 25° C. Fresh	Viscosity at 25° C. Stored for 24 hr. at 4° C.
	(%)	*(centipoises)*	*(centipoises)*
P =reconstituted nonfat dry milk[a]	11.24	33.9 (0)[b]	38.2 (−1.3)
2 =caseinate gel + water	7.32	5.3 (−0.2)	5.7 (−0.4)
3 =caseinate gel + Serum I	7.29	22.2 (+0.5)	38.0[c] (−4.1)
3H=3, heated ½ hr. to 85° C.	7.29	8.3 (+0.1)	13.6 (−1.4)
4 =caseinate gel + Serum II	8.42	8.4 (−0.1)	12.7 (−1.8)
4H=4, heated ½ hr. to 85° C.	8.42	5.7 (+0.1)	8.7 (−0.9)

[a] Low-heat.

[b] Values in parentheses: viscosity change after 15 min. in the viscometer.

[c] This concentrated caseinate dispersion formed a gel on storage for 24 hr. at 4° C., which thinned upon transfer and raise of temperature.

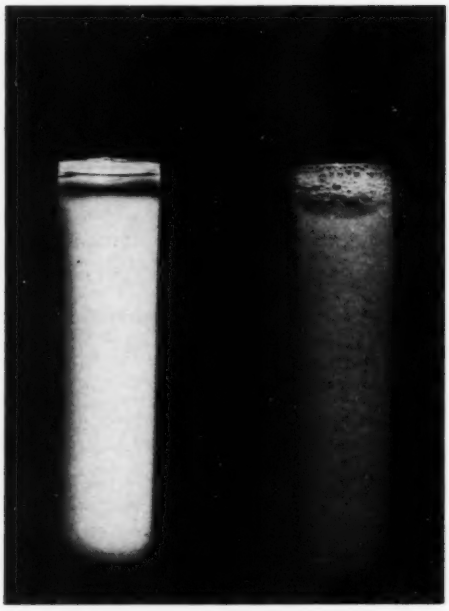

FIG. 5. Milkiness produced by heating the concentrated caseinate Model System 3 (Figures 3 and 4). Right tube, Concentrate 3 before heating. Left tube, same concentrate after heating (3H).

The present experiments merely demonstrate some effects obtained with electrolytes, to show that the caseinate model system opens a way for systematical study of the effect of various factors as pointed out in the introduction.

SUMMARY

A model system was prepared by redispersion of caseinate isolated from skimmilk (fluid or reconstituted from low-heat nonfat dry milk) by sedimentation in a supercentrifuge. A residual fraction of from five to ten per cent of the orignal casein remains in the supernatant. Caseinate was used as wet gel or in a dry form. Dry caseinate was obtained by freeze drying with elimination of shrinkage that results from local drying in the liquid state, which causes difficulty in dispersing.

The caseinate was dispersed to a colloidal state like that in skimmilk, by rotating an initial dispersion of caseinate in whey or water, in a bottle with beads at 4° C. or 60° C., and with air completely eliminated

A routine method for examining the state of dispersion was worked out, based on the rate of sedimentation in a small supercentrifuge.

Redispersion of caseinate in water resulted in the formation of a new residual fraction. Redispersion in artificial sera containing citrate, sodium, and calcium produced larger amounts of residual fraction, indicating a partial breakup of the complex, and partial formation of sodium caseinate.

Concentrated caseinate model systems in storage showed increase in viscosity, as the result of the formation of structure (gelation) like concentrated skimmilk, indicating that casein is the most important factor in the gelation phenomenon.

Concentrated caseinate Model System pH 7.25, containing 8.0 g. of citric acid, 4.3 g. of sodium hydroxide, and 0.4 g. of calcium per liter concentrate contributed by the artificial Serum I (in addition to the calcium and other ions contributed by the caseinate), was slightly "milky," but when heated produced a distinct milkiness that was reversible in storage. This can be explained by a change in equilibrium between sodium and calcium caseinate, owing to heat.

The caseinate model system opens a way for systematical study of the effect of various factors related to the physical and chemical changes that take place in milk products as a result of processing and storage.

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EFFECT OF MUMMIFIED FETUSES ON THE PROLIFICACY OF HOLSTEINS

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Breed and clinical records of 2,607 cows for 30 years, from a Holstein herd which was supervised by one man for 25 years, were used in this study. Mummified fetuses were produced by 1.1% of the cows, with gestations ranging from 131 to 342 days, involving 0.43% of the parturitions. These cows were above the herd average in reproductive periods and in average age. Heritability was estimated at 16% when service sire was excluded. Mummified fetuses resulted in 0.6% of the cases when bulls, postulated as heterozygous, were mated to daughters of other heterozygous bulls. There is no evidence that mummified fetus is transmitted as a single recessive in a simple Mendelian way. *Editor.*

A lethal condition in cattle, generally described as mummification, is characterized by a short neck, stiff legs, and prominent joints, and may be associated with muscle contracture and fetal resportion *(2, 5)*. A postulated cause of mummification *(1)* is placental hemorrhage which, if extensive, results in separation of fetal and maternal placenta. Torsion of the uterus is believed a frequent cause of failure to abort. Boyd *(1)* concluded from clinical study that fetal death occurs between the fourth and sixth months of pregnancy, with the fetus becoming progressively dry and hard with elapsed time.

Löje, as cited by Lerner *(3)*, describes mummification as a recessive lethal in Danish Red cattle.

SOURCE OF DATA

Breeding records, including clinical records maintained by resident veterinarians for a herd of Holsteins from 1920-1950 and under the supervision of one man since 1925, were utilized for this study. The information was coded and transferred to International Business Machine cards.

A method described by Lush *(4)* was used to estimate heritability. This method simply shows whether dams producing mummified fetuses have a higher incidence of daughters producing mummified fetuses than do dams observed and known not to have produced a mummified fetus.

RESULTS AND DISCUSSION

During the 30-year period, 2,607 cows with 7,387 observed parturitions and 9,994 reproductive periods were recorded (Table 1). Twenty-nine cows produced

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[2] Present address: Quaker Oats Co., Merchandise Mart, Chicago, Illinois.

32 mummified fetuses (three cows had two each), which represented 1.1% of the cows and 0.43% of the parturitions. These cows produced a mummified fetus one time in every 4.7 parturitions. Fourteen of the 29 cows failed to conceive again. These 14 cows averaged 8 yr., 4 mo. of age at the time the mummified fetus was delivered, and left the herd with an average of 4.9 parturitions per cow (herd average, 2.83). Fifteen cows subsequently calving again averaged 5

TABLE 1

Comparison of cows producing mummified fetus with the herd average for incidence and disposal rate by reproduction period

Reproductive periods in herd	Total cows calving	Mummified fetus		Total cows	Not completing reproductive period				Cows [a] in group
					Total cows		Cows having mummified fetus		
(No.)	*(No.)*	*(No.)*	*(%)*	*(No.)*	*(No.)*	*(%)*	*(No.)*	*(%)*	*(%)*
1	1,905	5	0.3	2,607	702	26.9	0	0.0	29
2	1,378	10	0.7	1,905	527	27.7	3	10.3	29
3	1,085	1	0.1	1,378	293	21.3	3	11.5	26
4	858	6	0.7	1,085	224	20.6	3	13.0	23
5	661	1	0.2	858	197	23.0	5	25.0	20
6	490	1	0.2	661	171	25.9	3	20.0	15
7	359	1	0.3	490	131	26.7	1	8.3	12
8	268	2	0.8	359	91	25.4	4	36.4	11
9	172	3	1.8	268	98	36.6	3	42.9	7
Over 9	211	2	1.0	383	173	45.2	4	57.1	7
Total	7,387	32	0.4	9,994	2,607	26.1	29	16.2	179

[a] Cows with mummified fetus—number in herd at each succeeding reproductive period, regardless of when mummified fetus was produced.

yr., 2 mo. at the time a mummified fetus was produced, and afterwards produced an average of 2.7 calves. These 15 cows averaged 5.5 total calvings each and left the herd at an average age of 10½ yr. (herd-average disposal age was approximately six yr. for all cows and 7.8 yr. for those not disposed of for dairy purposes). Only three of the 29 cows left the herd before 12 yr. of age because of sterility. Eleven were over 12 yr. old at the time of disposal. Thus, the cows producing mummified fetuses exhibited better than average longevity and reproductive performance.

The cows required 211 services for 80 conceptions (2.6 s/c) before conception which resulted in a mummified fetus. Mummified fetus conception required 2.7 services. Subsequent calvings averaged 2.9 services per conception (116 services and 40 conceptions). Since these latter reproductive periods occurred when the cows were older, no significance is attached to the increased services required for conception.

Gestation. It is generally accepted that death of a fetus which becomes mummified occurs most often during the fourth to the sixth month of pregnancy *(1)*. However, expulsion may be several months later and frequently under artificial circumstances. In this study, gestation averaged 215 days (range 131 to 342 days). The distribution of mummified fetus abortions by month of pregnancy was as follows: Less than six mo.—five; six mo.—eight; seven mo.—six; eight mo.

—four; nine mo.—four, and over nine mo.—four. One cow delivered a normal heifer and a mummified fetus at 9 yr., 9 mo., with a gestation of 283 days. This cow had previously produced a mummified fetus at 3 yr., 11 mo.

Other reproductive difficulties. Twenty of 27 cows had no recorded evidence of reproductive tract abnormalities after delivery of a mummified fetus, five showed infection, one was cystic, and one had a retained yellow body and anestrus for one year. Twinning rate was 6.0% for these cows, as compared with approximately 5.3% for all cows of comparable average age. Abortion rate, not counting mummified fetus, was 6.0% compared with 8.6% for all cows. Calves of cows producing mummified fetuses were 48.7% males, compared with 51.6% for all calves.

Heritability. The 29 cows which produced a mummified fetus left 45 daughters which had one or more reproductive periods in the herd. Two, or 4.4%, of these daughters produced a mummified fetus (Table 2). Dams not producing a mummified fetus left 2,031 daughters which reproduced one or more times. Twenty, or 1.0%, of these daughters produced a mummified fetus. The unadjusted chi-square value of 5.03 is significant at the three per cent level of probability.

TABLE 2

Daughters from dams producing and not producing mummified fetus

Minimum number of reproductive periods of dam and daughter	Dams producing mummified fetus		Dams not producing mummified fetus	
	Total No. daughters	Percentage producing mummified fetus	Total No. daughters	Percentage producing mummified fetus
1	16	0	644	0.5
2	8	0	411	0.5
3	7	14.3	253	0.4
4	2	0	190	2.6
5	4	25.0	158	0.6
6	0	0	124	0.8
7	2	0	85	2.4
Over 7	6	0	166	3.0
Total	45	4.4	2,031	1.0
Excluding Reproductive Periods 1 and 2	21	9.5	976	1.5

Dams and daughters would generally have unequal opportunity to produce a mummified fetus, because of the variable number of observed reproductive periods. Therefore, it appears appropriate to eliminate those daughter-dam pairs where one of the pair had less than three reproductive periods. Dams and daughters, each with a minimum of three reproductive periods (Table 2), showed 9.5% and 1.5% of the daughters producing mummified fetus, respectively, from dams producing and not producing mummified fetus. This difference is 8.0% percentage units. The unadjusted chi-square value of 6.9 is significant at the one per cent level of probability. The chi-square value mentioned here, as well as in the preceding paragraph, is unreliable, because only two daughter-dam pairs produced mummified fetuses. One less, or one more, observation would have changed the estimates drastically.

Estimation of heritability would depend on certain assumptions about the mechanism leading to production of mummified fetus. One possibility is that a dam and her mate each contribute genetic factors predisposing the daughter to produce a mummified fetus, under certain environmental circumstances independent of the daughter's mates.

If placental hemorrhage and torsion of the uterus *(1)* are major predisposing factors for mummification, then mummification should be independent of the sire involved in conception. Under this hypothesis, a proper estimate of heritability is twice the percentage difference between daughters of dams producing and not producing mummified fetus. This would yield an estimate of 16% (8% × 2) for heritability of genes acting in an additive manner. Though unbiased, this value for heritability is not very trustworthy, for reasons mentioned in the preceding paragraph.

If the daughter's mates also contributed to production of a mummified fetus, then a proper estimate of genes acting in an additive manner would be in excess of eight times the difference in the percentage of daughters from each type of dam. The factor would be eight, where the characteristic is solely one of the embryo, because relationship is that between half-niece and aunt. If mummified fetus was this type, the factor would be more than eight, because all progeny of a dam would not have equal opportunity to reproduce, as mummified fetus is lethal. The characteristics of the data do not support the latter assumption. Further, the incidence of mummification in the herd was very low and thus subject to a very large sampling error. Data are required from a much greater population of cows.

Incidence among paternal sisters. Löje, as cited by Lerner *(3)*, has indicated a single recessive. On this basis, the daugthers of bulls that are heterozygous for mummified fetus should produce a mummified fetus one time in eight when mated with other heterozygous bulls, and one time in four when heterozygous mates are involved.

The 29 cows which produced mummified fetus were sired by 25 different bulls, and their mates for conceptions terminating with mummified fetus involved 19 bulls. Detailed data for five bulls which sired daughters producing mummified fetus and which were also mates of cows producing mummified fetuses are shown (Table 3). These five bulls were also the most extensively used bulls.

Daughters of these bulls had 2,755 calvings, with 0.40% of the pregnancies mummified, as compared with an average herd rate of 0.43%. These daughters conceived 1,008 times to the service of the five bulls and mummified fetus was produced 0.60% of the time. The number of pregnancies and mummified fetuses for each bull, resulting from all matings to daughters of the other four bulls, is shown in the right half of Table 3. The interesting feature of this analysis is that these bulls averaged a slightly higher rate of mummification when all mates were considered (0.65%) then when only daughters of this group of bulls were used (0.60%). Eighty-one paternal brother-sister matings terminated in three cases of mummified fetus (3.7%). Since the standard deviation is about 2.1%, the comparison is interesting but not reliable.

TABLE 3

Incidence of mummified fetus among daughters and mates of five bulls postulated as heterozygous for mummified fetus

Bull code	Daughters			Granddaughters of other four bulls			Sire of conception					
										When mated to daughters of other four bulls		
	Total calvings	Mummified fetus		Total calvings	Mummified fetus		Total calvings	Mummified fetus		Total calvings	Mummified fetus	
(No.)	*(No.)*	*(No.)*	*(%)*	*(No.)*	*(No.)*	*(%)*	*(No.)*	*(No.)*	*(%)*	*(No.)*	*(No.)*	*(%)*
1	765	2	0.26	314	0	0	463	4	0.86	117	0	0
2	228	2	0.88	74	1	1.35	266	2	0.78	137	1	0.73
3	740	3	0.41	457	2	0.44	337	1	0.30	120	0	0
4	672	2	0.30	122	2	1.64	574	5	0.87	345	3	0.87
5	350	2	0.57	41	1	2.44	499	2	0.40	289	2	0.69
Total	2,755	11	0.40	1,008	6	0.60	2,139	14	0.65	1,008	6	0.60

In further interpreting the results of Table 3, it is of interest to know that Bull 1 was the sire of Bull 2 and the maternal grandsire of Bulls 4 and 5. The sire of Bull 3 was a grandsire and a great-grandsire of Bulls 2, 4, and 5. Bull 4 was the sire of Bull 5.

General discussion. There is no evidence in these data that mummified fetus is transmitted as a single lethal recessive in a simple Mendelian manner. Lack of evidence for a genetic mechanism for mummified fetus is understandable, since mummification is either resorption or abortion failure. Further, as explained by Wright *(6)*, under circumstances where recessive alleles are rare, or where the genes also belong to an interaction system, the correlation between parent and offspring approaches zero, even without environmental interference. Environmental influence is indicated, because the time of fetal death varies and a mummified fetus may not be observed for months. Occurrence of mummified fetus suggests other factors, such as pathological conditions of the uterus at the time of fetal death, age of fetus, torsion of uterus, and the functional state of the yellow body of pregnancy. In addition, several lethal genetic factors not easily recognized or observed because of resorption, early abortion, or mummification could also conceivably be involved.

SUMMARY

Over a 30-year period in a closely observed breeding herd, 29 of 2,607 cows produced 32 mummified fetuses. This represented 1.1% of the cows and 0.43% of the parturitions. The range in gestation was 131 to 342 days (mean = 215 days). Cows having mummified fetus were considerably above herd average in number of reproductive periods and in average age at disposal. Heritability on an additively genetic basis was estimated at 16% when service sire was assumed not to be a factor. Matings of bulls postulated as heterozygous to daughters of other heterozygous bulls (1,008 cases) terminated in mummification in 0.60% of the cases. These data show that mummified fetus is not transmitted as a single lethal recessive in a simple Mendelian manner.

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DRY WHOLE MILK. I. A NEW PHYSICAL FORM

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This paper reports the properties of a new dry whole milk, dried under high vacuum and low temperatures in the form of an expanded sponge-like structure. Milk is homogenized at 145° F. and 2,500 p.s.i. and pasteurized at 162° F. for 16 seconds. It is concentrated to 47 to 50% total solids at 85° to 100° F. in a high-vacuum falling film evaporator, heated to 135° F. and homogenized at 4,000 and 500 p.s.i. Before the concentrate is homogenized, nitrogen is entrained in it and it is then flowed over drying pans, cooled to 55° F., and vacuum-dried as a foam. Finally, the dried mass is crushed lightly. This product disperses easily in cold water and has a natural flavor when reconstituted in the fresh state. *Editor.*

A long-range research program was recently initiated at this Laboratory, with the ultimate goal of developing a dried whole milk of easy dispersibility, good flavor, and storage stability. As presently conceived, this program will be carried forward in three phases: The first phase will be concerned with the preparation by batch methods of a dried whole milk which is initially easy to disperse and of good flavor. The second phase will include a study of the storage stability of the material and factors which influence stability. The third phase will be a translation of the essential processing conditions to a commercially feasible operation. The purpose of this paper, which deals with the first phase of the program, is to report the properties of whole milk dried in a new physical form and possessing excellent flavor and easy dispersibility in ice water. The processing variables found to be essential in its preparation are discussed.

CHOICE OF THE DRYING METHOD

Puff-drying methods were investigated, because the authors believe them to be superior to spray- or roller-drying. Puff-drying has been successfully applied to heat-sensitive citrus juices and other food products in both batch and continuous processes *(2-5, 7)*. Therefore, a systematic engineering study was made of the factors responsible for producing a product that would be easy to disperse and have good flavor.

In general, puff-drying may be defined as the formation of a highly expanded, sponge-like structure of dried material from a thin film of liquid concentrate, under conditions of high vacuum and low temperature. The product would be expected to disperse rapidly because of its large surface area per unit weight, and to possess natural flavor because of the high-vacuum, low-temperature drying conditions employed. As applied to whole milk, drying at low temperatures

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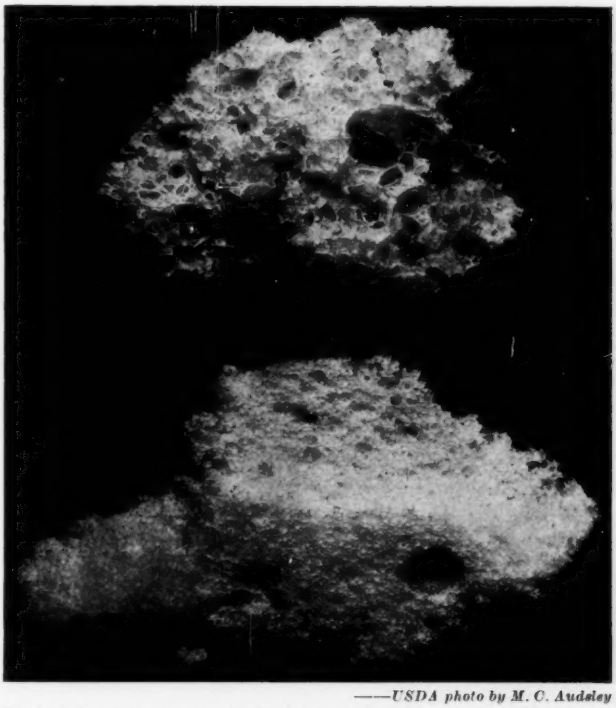
would also be expected to inhibit protein destabilization, and dehydrating at low oxygen concentrations at low temperature would preclude atmospheric oxidation.

BASIC CONSIDERATIONS IN PUFF-DRYING WHOLE MILK

Puffed structures can be developed by various devices and, for some materials, such as those containing large amounts of sugars, the type of structure may not be of prime importance for rapid dispersibility. With whole milk, however, the structure has been found to influence markedly the dispersibility rate of the dried product. Two possible structures are illustrated (Figure 1). The puffed form on the top was developed by the evolution of water-vapor from a deaerated concentrate. This is characterized by large, nonuniform bubbles and a preponderance of unpuffed, intercellular material. Product obtained from such a form disperses very poorly compared to that shown (bottom of Figure 1). The latter was formed by the expansion of entrained gas of low solubility in the concentrated milk, and is characterized by small, uniform bubbles and a minimum of unexpanded intercellular material. To differentiate, the latter is hereafter referred to as a foam.



——USDA photo by M. C. Audsley

FIG. 1. Typical foam structures obtained by water-vapor (top) and entrained gas of low solubility (bottom).

The mere use of an entrained gas does not insure a form that will disperse readily. In order for entrained gases to be effective in forming a fine-grained foam, the concentrate must be held at a low temperature during the initial drying stage. At temperatures above 55° F., and under the vacuums necessary for foaming, the concentrate will boil before it has expanded sufficiently, and boiling at this point will remove the gas from the unexpanded material. The dried structure will then be similar to a puff formed by water-vapor from a deaerated concentrate. Initial experiments using only dissolved CO_2 or N_2O indicate that these gases, because of their high solubility, give structures similar to the water-vapor type.

When the absolute pressure within the drier reaches the vapor pressure of the concentrate, vigorous agitation of the expanded structure results from the flashing of water-vapor. If the desirable form is to survive, it must be rigid enough to withstand this action. Here again, low temperatures are desirable because of the added viscosity. High solids content is another factor which increases the viscosity of the concentrate, and it has been found that materials containing up to about 50% solids can be readily dried as a foam. Beyond this concentration a gel forms which becomes difficult to spread evenly for drying.

The conditions essential for producing a material of good dispersibility have been determined by experiments in a pilot-plant vacuum shelf drier. Exploratory studies conducted cooperatively with the Chain-Belt Company [2] of Milwaukee, Wisconsin, have shown, moreover, that these essential conditions can most probably be translated to a continuous method and thus, that foam-drying of whole milk may have a future in commerce. A public service patent is pending covering these principles.

PREPARATION OF THE DRIED WHOLE MILK

Fresh pasteurized, homogenized milk was obtained from a local dairy for these studies. It had been homogenized at 145° F. and 2,500 p.s.i. and then pasteurized at 162° F. for 16 seconds. It contained 3.6 to 3.7% fat and about 8.7% solids-not-fat. The milk was first concentrated to from 47 to 50% total solids at a batch temperature of 85 to 100° F. in a high vacuum, falling-film evaporator. It was then heated to 135° F. and homogenized, first at 4,000 p.s.i. and then at 500 p.s.i., through a single-stage homogenizer of the pulsator type. Immediately prior to homogenization, nitrogen was bubbled through the concentrate from a fritted-glass sparger dispersing entrained gas through the material. The concentrated milk was then flowed over stainless steel drying pans to an average depth of 1/16-inch, chilled to 55° F. or below, and dried as a foam in a vacuum shelf drier. The resulting dried mass was crushed lightly through stainless steel screens. A typical drying cycle is illustrated (Figure 2).

[2] Mention of companies and commercial products anywhere in the paper does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

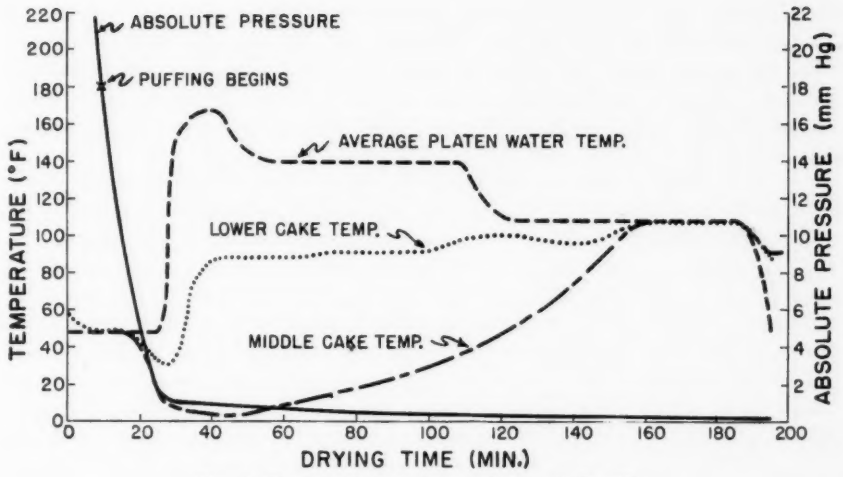


FIG. 2. Vacuum shelf drying cycle for whole milk.

It has been found that variations in foam structure were possible, depending on the rate of vacuum application. For instance, when the pressure inside the drying chamber was reduced from 20 to 2 mm. in five minutes, the concentrate expanded rapidly to about 24 times its original volume, and then became sufficiently rigid to prevent further expansion or collapse. The resulting structure was composed of tiny bubbles approximately 1/16-inch in diameter and had a relatively large proportion of unexpanded intercellular material. When the drying chamber pressure was reduced from 20 to 2 mm. in about 20 minutes, on the other hand, the concentrate expanded slowly to a volumetric increase of from 32- to 96-fold before it stabilized. Bubble sizes were larger in these structures, and the proportion of unexpanded intercellular material was substantially reduced. Where slow-vacuum application was used, variations in volume appeared to be caused by the viscosity of the concentrate. In general, concentrates of higher viscosity contained more entrained gas and expanded to a greater extent. Where vacuum was applied rapidly, on the other hand, concentrates of different viscosities all expanded to about the same degree, indicating that in those cases the rapid application of vacuum was the controlling variable.

RATE-OF-DISPERSIBILITY TEST

Because the structure could be altered by varying the degree of expansion of the concentrate, a reliable method was required for determining the rates at which the several products would disperse. To fill this need, the ease-of-dispersion method of Stone *et al. (6)* was studied and modified. In the original method, 15 gm. of material were added to 90 ml. of water at from 70 to 75° F. in a 250-ml. beaker. The mixture was stirred by hand with a teaspoon for a designated time-interval, dispersing as much of the material as possible without

splashing the dried milk or water out of the beaker. After stirring, the mixture was filtered through a coarse (40 to 60 microns) fritted-glass Buchner funnel, with the aid of about 16 inches of vacuum. The filtrate was then diluted to 200 ml. and grams of total solids were determined in a 20-ml. aliquot. When multiplied by ten, this weight represented the dispersion value for the corresponding stirring time.

Several modifications in this test were made, in an effort to more nearly standardize the filtration step from one material to another, to detect smaller differences among dried milks, and to improve the precision of the method. In the original test it was found that some materials bound the fritted-glass filter, whereas others did not. This condition, in turn, varied the holdup time in filtering from a few seconds for readily dispersible materials to more than two minutes for materials of poorer dispersibility. This wide variation is obviously undesirable. Two modifications in the procedure brought the filtration time for all materials to within about three to 15 seconds. The first was the use of scalper filters, consisting of cones of 100-mesh screen (140 microns) and 150-mesh screen (103 microns), just ahead of the vacuum filter to eliminate filter binding. The second provided that instead of collecting all the filtrate for analysis, only 35 ml. should be used.

Collecting only the first 35 ml. of filtrate for analysis improved the precision of the method in two other ways. First, it was questionable that all of the filtrate could have been collected, and second, the initial fraction of the filtrate was probably more representative of the solution immediately after stirring than the total filtrate. Thus, by removing only the minimum of filtrate necessary for analysis, the solution was separated from the undispersed material more quickly, and any further dispersing of solids after stirring was minimized.

Danger of splashing during stirring was reduced by substituting a 300-ml. tall-form beaker for the 250-ml. beaker used in the original test.

In order to detect smaller differences among dried milks, the filter screens and beaker were chilled in ice water prior to use and the temperature of the stirring water was reduced to 38° F. The variation in dispersibility caused by changing the water temperature from 75 to 38° F. is illustrated (Figure 3). For this experiment, materials from the same respective batches were used. A comparison of the curves at the two temperatures for either material illustrates the effect of temperature on dispersibility rate, and a comparison of the curves for the two materials at 38° F. and then at 75° F. illustrates that smaller differences in dispersibility can be detected by using water at 38° F.

In summation, the rate-of-dispersibility test as used in this work was as follows: Fifteen grams of powder at room temperature were added to 90 ml. of water at 38° F. in a 300-ml. tall-form beaker. The mixture was stirred with a teaspoon for a predetermined time-interval, dispersing as much of the powder as possible without splashing. The mixture was then poured through a 100-mesh cone contained in a glass funnel, dropped through a 150-mesh cone contained in another glass funnel, and finally dropped onto a coarse fritted-glass Buchner

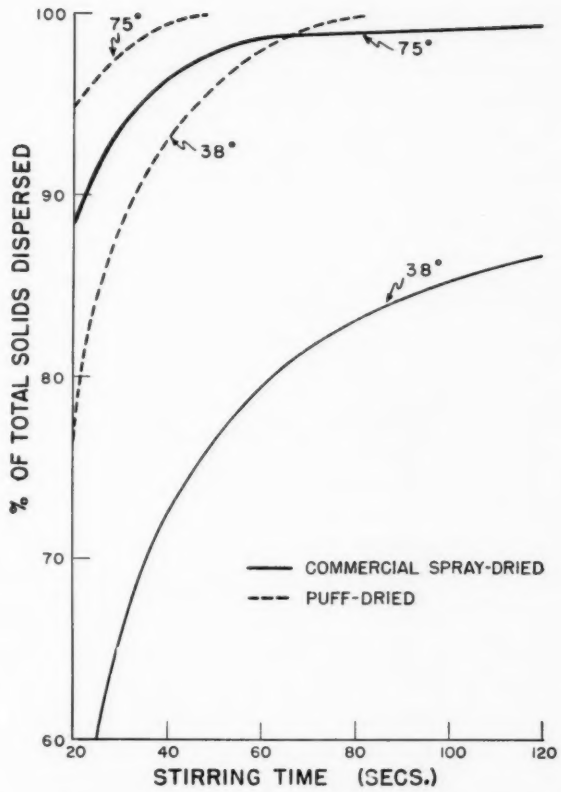


FIG. 3. Dispersibility rates for dried whole milk and effect of water temperature.

suction filter with about 16 inches of vacuum on the filtering flask. After 35 ml. of filtrate had been collected, the filtration was discontinued. An aliquot of 25 ml. was added to an aluminum pan of known weight and concentrated to a plastic state in a Brabender Moisture Tester,[2] using an air current at 275° F. The material was then brought to dryness overnight, in a vacuum oven operating at about 194° F., 0.4-inch absolute pressure, and with a bleed of dry air. Percentage moisture in the filtrate was then determined, and dispersibility was calculated as the percentage of the total solids in the dried milk which had dispersed sufficiently in the given time to pass through the filters. This calculation was carried out as follows:

Let x = % solids of the dried milk
y = % moisture of the filtrate
F = Grams of filterable solution (assuming the 35 ml. collected was representative)

G = Grams of solids contained in F
T = Grams of solids m.f.b in the 15-gram sample
S = % of the total solids in the dried milk that had dispersed

Then, $F = (90/y)\ 100$

$G = F - 90$

and $T = \frac{15 \times}{100}$

Thus, $S = \frac{G}{T} \times 100$

Six stirring times were employed and each stirring time was run in triplicate. The filtering system is sketched (Figure 4).

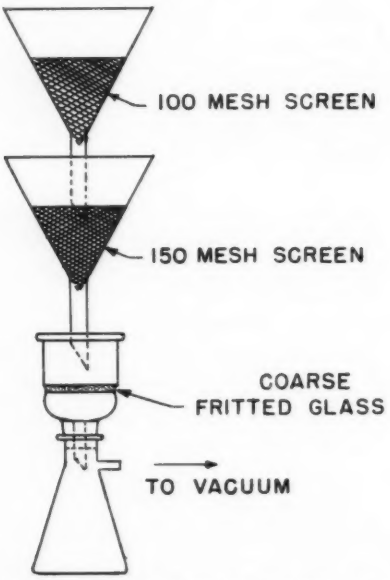


FIG. 4. Filtering system for dispersibility-rate test.

In an experiment designed to test the precision of the dispersibility-rate procedure used in this work, three different dried milks were run through the dispersibility-rate test in random order, using stirring times of 20, 40, 60, 80, 100, and 120 seconds. The data (see Table 1) for solids content of the filtrates were analyzed statistically, using the analysis of variance technique.

The coefficient of variability for this experiment was 1.88%. Differences between treatment means of 0.22% solids were significant at the 0.01 level of probability. Expressed as percentage of the total solids in the dried milk which have dispersed, a mean difference of 0.22% solids in the filtrate corresponds to plus or minus 1.8% total solids at the point of maximum dispersion. Thus, this is a precise method for measuring rates of dispersibility.

TABLE 1

Solids contents of dried whole milk filtrates, as measure of dispersibility rate

Material	A	B	C
Stirring time *(sec.)*		Total solids[a]	
20	10.03	10.31	10.26
40	12.03	12.27	12.47
60	12.46	13.11	13.11
80	12.67	13.45	13.50
100	13.13	13.51	13.67
120	12.97	13.87	13.65

[a] Mean of three replicates.

DISPERSIBILITY RATES OF FOAM-DRIED WHOLE MILKS

Batches of material of the various types of foam structure developed by entrained gas were crushed lightly through 20-mesh (0.034-inch) and 40-mesh (0.015-inch) screens, and dispersibility rates were determined, using the modified test described above. Data from these tests are shown (Table 2).

Statistical comparisons of the data were made, using the analysis of variance technique. These showed that 20-mesh screening was superior to 40-mesh in all cases, that there was no real difference apparent between 32- and 72-fold expansion, that 96-fold expansion was poorer than 32- and 72-folds, that 24-fold expansion was poorer than the other three, and that differences caused by degree of expansion, screen size, and stirring time were all significant beyond the 0.01 probability level. In essence, these results show that the optimum dispersibility rate for foam-dried material is unaffected by the degree of expansion of the concentrate over the wide range from 32-fold to at least 72-fold; that there are, however, upper and lower limits, and that 20-mesh screening is more desirable than 40-mesh.

BULK DENSITIES OF FOAM-DRIED WHOLE MILKS

Bulk densities of the materials listed in Table 2 were determined, in order to exhibit the effects of volumetric expansion and screen size. These data were found by carefully placing 100 ml. of material in a 250-ml. graduated cylinder, weighing the material, and calculating its bulk density untamped. Then the graduate was tapped briskly upon a table top, until the material reached a con-

TABLE 2

Mean percentage dispersibilities of dried whole milks

Volumetric expansion (fold)	24		32		72		96	
Screen size (mesh)	20	40	20	40	20	40	20	40
Stirring time *(sec.)*	Total solids							
10	41.0	20.7	54.8	35.6	49.0	24.6	—	—
25	71.6	59.3	83.6	67.4	80.2	69.8	79.7	70.2
40	83.9	79.3	92.2	81.0	88.7	83.5	91.4	80.0
55	91.5	85.2	97.2	90.2	96.3	90.0	89.2	85.0
70	93.4	89.0	99.2	92.8	96.2	91.0	96.2	92.8
120	97.2	97.2	101.9	99.9	100.1	98.0	98.9	99.5

TABLE 3

Bulk densities of dried whole milks, gm. per cc.

Volumetric expansion (fold)	24		32		72		96		Commercial product[a]
Screen size (mesh)	20	40	20	40	20	40	20	40	
	Density								
Untamped	0.17	0.19	0.18	0.30	0.15	0.20	0.13	0.28	0.54
Tamped	0.27	0.34	0.25	0.42	0.25	0.35	0.22	0.50	0.72

[a] A spray-dried product available commercially.

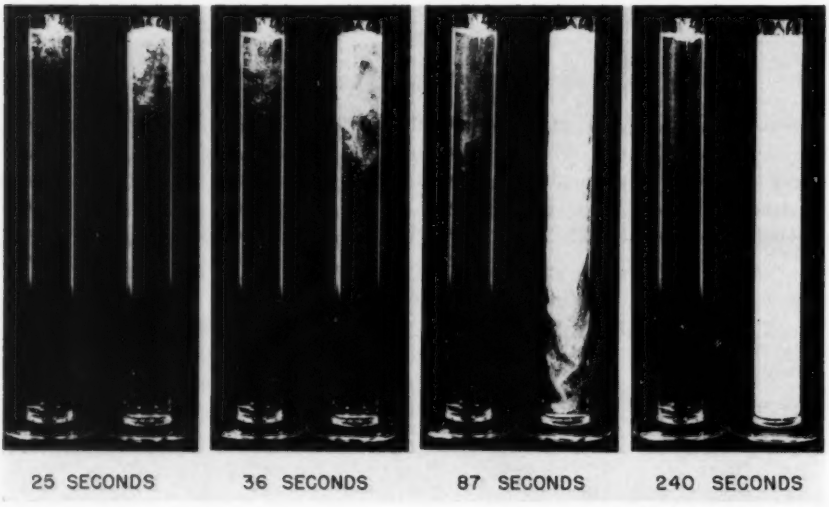
stant level in the graduate, and its new volume was determined as a basis for calculating its bulk density tamped. These data are shown (Table 3).

There is no consistent trend in these data which would indicate a variation of bulk density with degree of expansion of the concentrate, either at 20- or 40-mesh screening. As would be expected, however, the powders run through the 20-mesh screen are more bulky than those run through the 40-mesh screen. Assuming an averaged tamped bulk density of 0.25 gm/cc for 20-mesh, and 0.43 gm/cc for 40-mesh, a unit volume of material would yield 2 and 3½ volumes, respectively, of reconstituted fluid milk.

OTHER CHARACTERISTICS OF FOAM-DRIED WHOLE MILKS

Foam-dried whole milks contained only trace amounts of undispersed material when run through the solubility index test *(1)*.

Comparisons of the whey proteins of fresh whole milk and freshly prepared



——*USDA photo by M. C. Audsley*

FIG. 5. Comparison of self-dispersing characteristics of foam-dried and spray-dried whole milks in cold water.

foam-dried material were made. In these studies, the dried wilk was reconstituted to three times the concentration of fluid milk, the fat separated by centrifugation, the casein precipitated by acidification, the resulting whey solution dialyzed against a phosphate buffer solution of pH 7.5, and ultracentrifugal inspection carried out at 25° C. The fresh milk was treated similarly, except that concentration of the material was carried out after the casein had been precipitated. Ultracentrifugal patterns showed no difference between the whey proteins of the fresh milk and the foam-dried material.

A striking demonstration of the ease of dispersion of a foam-dried whole milk is given (Figure 5). For this test, two 250-ml. clear, graduated cylinders were filled with water of about 38° F. The water surface of the right-hand graduate was carefully covered with foam-dried material and the other with a spray-dried commercial product. Photographs were taken after 25, 36, 87, and 240 seconds. The superior dispersing characteristics of the foam-dried whole milk are evident.

SUMMARY

A dried whole milk of excellent fresh flavor and rapid dispersibility in cold water has been prepared in a vacuum shelf drier, using special foam-drying techniques. The important variables studied in the batchwise drier can probably be translated to a continuous method.

A modified test for determining the rates at which dried whole milks disperse has been shown to have good precision. Using this test, foam-dried whole milks will completely disperse in water of 38° F. within 100 seconds with manual stirring, and in water of 75° F. within 50 seconds.

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STUDIES ON STARTER METABOLISM. I. THE RELATIONSHIP BETWEEN STARTER ACTIVITY AND THE PREDRYING HEAT HISTORY OF RECONSTITUTED NONFAT DRY MILK SOLIDS[1,2]

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The objective of this work was to determine the effects of heat on the suitability of nonfat dry milk solids (NDMS) for starters. In the absence of additional heat treatment beyond that provided in manufacturing it was found that high heat powders produced better starters than the low heat ones, as indicated by faster resazurin reduction times and increased acid production. However, NDMS powders with a severe heat history had a deleterious effect on starter activity. *Editor.*

Milk can often serve as a complete and unsupplemented nutrient medium for the growth and activity of lactic acid bacteria. However, certain manufacturing processes, especially heating, can alter this product, rendering it stimulatory in some cases and inhibitory in others. The dried milk industry is especially interested in the effect of heating milk on starter activity. During manufacture, the original milk is subject to various heat exposures during pasteurization, preheating, condensing, and drying. Since the cumulative effect of these heat treatments could influence the development of starters in the reconstituted milk, and since in 1954 alone over 35,000,000 lb. of nonfat dry milk solids (NDMS) were employed in cheese and cultured buttermilk manufacture *(1)*, this problem becomes very important.

Horrall and Elliker *(7)* reported that reconstituted milk promoted more constant activity in starters than did selected whole milks. On the other hand, different lots of NDMS varied in ability to provide a satisfactory medium. Since no data were presented regarding the history of the powders, it was difficult to ascertain whether the manufacturing process or the original source of milk was responsible for the variation. In further work, Anderson and Elliker *(3)* found that the addition of individual amino acids and/or vitamins to low-heat NDMS produced no marked stimulation in the growth of individual lactic streptococci or mixed-strain starter cultures. The NDMS consequently contained the minimal requirements essential for growth. Marked stimulation, however, was observed when liver fraction L, trypsinized skimmilk, or peptonized milk were added in low concentration. This result was attributed to a peptide, or

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peptide-like, fraction similar to strepogenin. Further evidence for the stimulatory nature of milk peptides was provided by Anderson *et al. (4)*.

Rudnick *(9)*, in a study of NDMS utilization for cottage cheese manufacture, observed that NDMS with from 20 to 30% of the serum protein denatured by heat supported most rapid acid production. Other reports *(5, 8)* also recommended the use of low-heat NDMS for cottage cheese manufacture, but there was no specification as to what constituted a low-heat treatment.

In general, it might be stated that despite the widespread use of NDMS for starter propagation and cottage cheese making, relatively few reports have appeared on systematic studies on the suitability of different powders for this purpose. In particular, many problems associated with the influence of manufacturing processes on this medium have not been solved. During a series of investigations on the response of starter cultures to the heat treatment of the substrate, a number of trials were performed using NDMS samples with different heat-treatment histories. This paper presents a summary of some of these trials.

EXPERIMENTAL PROCEDURE

The starters employed were typical mixed strains of lactic streptococci and associated flavor-producing organisms normally used in the production of cheese and cultured buttermilk. They were routinely propagated at 20° C. (16-hour incubation) in selected whole milk which had been steamed for one hour. Immediately prior to a starter activity test, the starters were passed through a routine transfer sequence, in order to provide inocula that contained essentially the same number of organisms of the same physiological age for each of the various trials.

A satisfactory starter activity test was developed, based on acid development and resazurin-dye reduction. This test gave easily reproducible results and consisted of the following steps: *(1)* All samples of milk were dispensed into pyrex-glass bottles in either 50- or 100-ml. quantities, and were tempered in a controlled water-bath to 25° C. *(2)* At one-minute intervals, the samples were inoculated with one per cent of a 22- to 24-hour culture, which had been propagated at 20° C. in a uniform lot of reconstituted NDMS (nine per cent solids). *(3)* The samples were swirled to disperse the inoculum, covered with aluminum foil, and incubated at 25° for exactly eight hours. *(4)* At the end of the incubation period and at one-minute intervals, a nine-ml. aliquot of each individual sample was titrated with 0.1 *N* NaOH to the phenolphthalein end-point. *(5)* Uninoculated samples incubated under the same conditions served as controls. *(6)* Ten ml. from each tempered, inoculated sample from Step 2 was removed immediately after the starter was added and was dispensed into an individual screw-cap test tube, immersed in a refrigerated water-bath at less than 5° C. *(7)* When aliquots of each sample had been dispensed, one ml. of a standard resazurin solution *(2)* was added to each tube. *(8)* The dye and milk were mixed by slowly inverting the rack and were tempered to 30° C. in a water-bath. *(9)* After the proper incubation temperature had been reached, the samples were in-

verted and observed at 15-minute intervals until complete reduction to white had occurred.

In order to evaluate the activity of starters in a series of NDMS samples representative of the wide range of commercial powders available in this country, 80 samples of powder purchased in 13 states were tested. The heat histories of these milk powders were evaluated by the Harland-Ashworth serum protein method *(6)*. The samples were reconstituted at room temperature in distilled water (ten gm. powder in 100 ml. milk) by agitation with a stainless steel hand-mixer. After reconstitution, the samples were tempered and subjected to the activity tests outlined above.

Since the precise heat histories of commercial powders cannot be ascertained, several lots of powder were manufactured in the University pilot plant, where exposures could be carefully controlled and recorded. Skimmilk was preheated to the desired temperature in ten-gallon cans and was concentrated to 40% solids in a 16-inch Rogers vacuum pan. Drying was accomplished in a special experimental drier *(10)*, where the inlet-air ranged from 177 to 226° C. and exit-air temperature was held between 76 and 83° C. This procedure is essentially a standard operation that would be employed in commercial dry-milk manufacture. Samples of the raw milk, preheated milk, condensed milk, and finished powder were taken during the trial and were subjected to starter activity tests, after reconstitution with distilled water to the total solids content of the original milk.

RESULTS

A survey of commercial NDMS samples from different parts of the country lent support to the belief that the heat history of milk plays an important role in starter activity. Responses of two starters are plotted against the relative intensity of heat treatment (Figures 1 and 2). The latter criterion was not absolute, because there is no direct relationship between heat treatment and amount of undenatured serum protein. Actually, given heat treatments result in fairly reproducible percentages of denatured serum protein. However, if the original concentration of serum protein in the fluid milk is not known, it is not possible to ascertain the temperature history. Nevertheless, it was felt that with a large number of samples, it would be justifiable to assume an average value of 10 mgm/gm serum protein nitrogen. It would then be possible to rank the samples as relatively high- or relatively low-heat powders according to the undenatured serum protein remaining.

On the basis of this assumption, inspection of Figure 1 reveals that there is a rather wide discrepancy among different NDMS samples, insofar as their ability to support starter activity is concerned. However, this variation is particularly evident in those samples having more than five mgm/gm undenatured serum proteins, or according to the above-mentioned premise, those powders with a relatively low heat history. On the other hand, milks heated to higher temperatures provided a more uniform substrate with little variation in reduction time. Furthermore, the high-heat samples supported faster reduction times, indicating better conditions for starter activity.

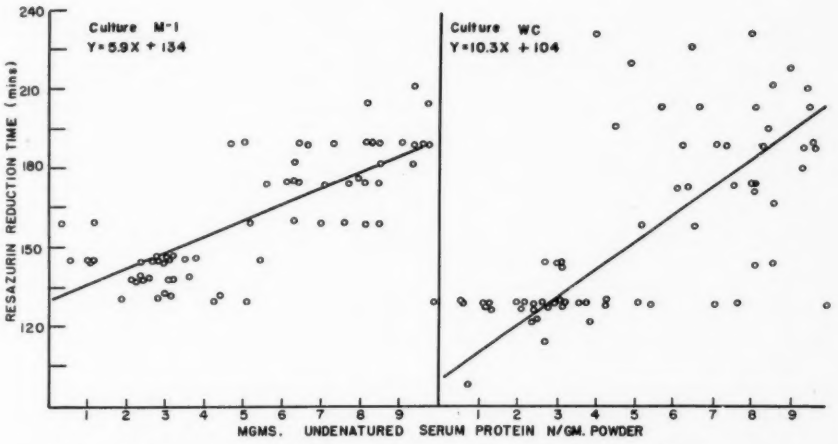
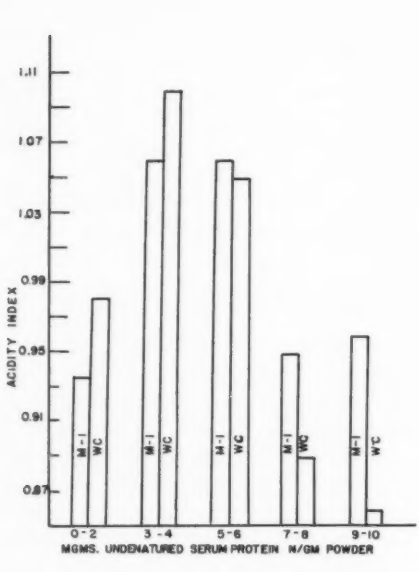


FIG. 1. Influence of heat treatment as measured by serum protein denaturation on resazurin-reduction time by starters in reconstituted NDMS (one per cent inoculum; 30° C. incubation).

Similar phenomena are illustrated (Figure 2), where the acidity index (acid produced by a culture under standard conditions compared to a reference powder tested under the same conditions) is plotted against relative heat treatment (estimated by denatured serum protein). In order to simplify the chart, samples were grouped into five categories of relative heat treatments and an average acidity index was calculated for each category. Milks with a low-temperature history (from 7 to 10 mg/gm) supported less acid development than did milks of higher heat treatment (from 3 to 6 mg/gm). On the other hand, rather intense heating which denatured close to 100% of the serum protein apparently inhibited starter activity in the resulting powder.

The importance of heat history in the response of starters in reconstituted NDMS is further illustrated (Table 1). Both powders were prepared from the same lot of fluid skimmilk, but had been subjected to a different heat treatment before condensing or drying. These data show that although there is a marked difference in the activity of nine different starters, high-heat NDMS served as a better medium for dye reduction than did the low-heat sample. However, it is necessary to point out that even in the uninoculated controls, high-heat NDMS reduced resazurin dye faster. The extent of reduction attributable to the milk constituents themselves could not be ascertained satisfactorily. Consequently, another comparative pair of NDMS samples was studied using acid production as the criterion of starter activity. These results (Table 2) further substantiate the observation that high-heat NDMS is a better substrate than low-heat NDMS. Though some of the differences do not exceed the limits of experimental error involved in sampling and titrating, a trend was clearly established and the results are reproducible.



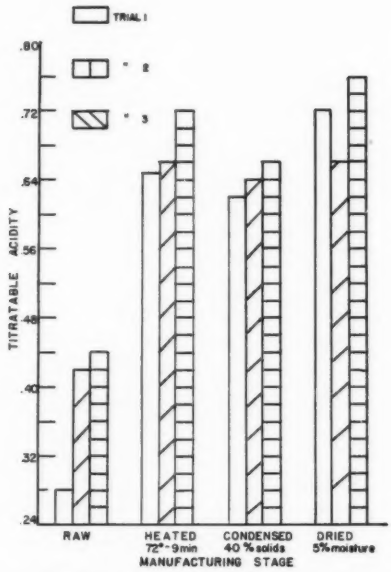


FIG. 2. Influence of heat treatment as measured by serum protein denaturation on acid production by starters in reconstituted NDMS. (Acidity index = titratable acidity produced by a one per cent inoculum after eight hr. at 25° C., compared to activity in a standard sample of NDMS.)

FIG. 3. Effect of heating during different stages in NDMS manufacture on starter activity. (Culture WC; one per cent inoculum; 18 hr. of incubation at 20° C.)

It was of interest to determine whether the response of starters in these products was owing to the preheat treatment, as assumed, or whether the other processes which involved heating overshadowed this original exposure. Results

TABLE 1

Activity (resazurin-reduction time in minutes) of various starters incubated at three different temperatures, in reconstituted NDMS with different predrying heat histories

Starter culture (1% inoculum)	37° C.		30° C.		20° C.	
	Low-heat[a] NDMS	High-heat[b] NDMS	Low-heat NDMS	High-heat NDMS	Low-heat NDMS	High-heat NDMS
HD48	150	150	157	120	420	300
LOL	120	120	150	120	420	300
M1	180	120	157	90	420	210
WC	120	120	120	90	300	270
169	120	90	120	90	300	270
M-2	120	90	90	90	390	180
125	150	150	150	120	420	270
184	150	150	165	120	420	270
H	180	165	120	90	300	270
Control	390	300	570	420	720	720

[a] Low-heat NDMS — preheat treatment of 72° C. — 9 min.
[b] High-heat NDMS — preheat treatment of 83° C. — 30 min.

TABLE 2

Starter activity (titratable acidity produced in 8 hr. at 25° C.) of ten different starter cultures when grown in high- and low-heat NDMS

Starter culture (1% inoculum)	Low-heat NDMS (63° C. — 30 min.)	High-heat NDMS (80° C. — 30 min.)
WC	0.52	0.57
HD48	0.39	0.49
184	0.48	0.53
NZK	0.31	0.33
LOL	0.50	0.56
M-2	0.49	0.56
125	0.54	0.61
H	0.51	0.54
169	0.50	0.53
M-1	0.50	0.53
Control	0.18	0.18

of these typical trials are presented (Figure 3). It can be seen that the major change in starter activity resulted from the preheat treatment, and that condensing and drying had a comparatively minor effect. Since these trials were carried out with precautions to avoid excessive heat treatment during the condensing and drying steps, it should be noted, therefore, that in manufacturing plants where these precautions are not observed the heat applied during all steps could mask the preheat treatment, and that material labelled as low-heat powder could, in reality, elicit the response of high-heat powder.

DISCUSSION

Milk-powder manufacturers, in particular those who supply NDMS for cultured product uses, should be aware of the relationships illustrated in this investigation; namely, that starter responses can be closely related to the relative intensity of heat treatment provided to milk powders during manufacture. Furthermore, it might be of interest to note that under careful manufacturing conditions it was possible for starter response to a powder to be the effect of the preheat treatment rather than the other manufacturing steps. In practical terms, this means that a manufacturer could actually supply his customers with uniform lots of NDMS designed for the best starter activity, as well as powder designed for optional body and texture characteristics in the finished product.

It is common practice for powder manufacturers to evaluate their products in regard to their freedom from antibiotics or other materials that might inhibit starters. Data are now available to suggest that heat treatment also be used as a criterion for starter-culture suitability. Furthermore, the discrepancy between resazurin-reduction activity tests now commonly employed and the acid-production test, might indicate the need to employ the latter in a true evaluation of the powder. It would be necessary to establish a carefully controlled preheat treatment that would yield the optimal powder for a given set of manufacturing conditions, and it would be necessary to control condensing and drying operations, in order to avoid exposures which could cause a heat-induced inhibition, such as was observed in the case of the high-heat samples (Figure 2).

SUMMARY

The predrying heat history of nonfat dry milk solids was shown to have a marked influence on the subsequent activity of starters propagated in that milk after reconstitution. In general, commercial high-heat powders supported starter activity more favorably than did low-heat powders, and this effect could be illustrated both by acid production and speed of dye reduction. However, NDMS samples with an excessively severe heat history exerted a deleterious effect on starter activity. It was possible to establish that the response of cultures to the heat history of milk-powder samples was not an isolated occurrence, but rather was a general phenomenon for a wide variety of starters. Furthermore, the response of starters could be related rather closely to the heat treatment of the milk before condensing and drying. The practical implications of these results are discussed.

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STUDIES ON STARTER METABOLISM. II. THE INFLUENCE OF HEATING MILK ON THE SUBSEQUENT RESPONSE OF STARTER CULTURES[1,2]

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The responses of starters were compared in fluid milks with different heat histories. Progressively severe individual heat treatments ranging from 62° C. for 30 minutes to 120° C. for 60 minutes resulted in a series of related changes in starter activity. A primary zone of stimulation was observed from 62° C. for 30 minutes to 72° C. for 40 minutes, and was followed by a zone of inhibition which began at 72° C. for 45 minutes and lasted through 82° C. for 10 to 120 minutes and 90° C. for 1 to 45 minutes. A second zone of stimulation began at 90° C. for 60 to 180 minutes and autoclaving at 120° C. for 15 to 30 minutes. Final inhibition occurred by autoclaving for more than 30 minutes. The first and second phases of the stimulation-inhibition-stimulation cycle were simulated by adding denatured serum protein or cysteine hydrochloride. In raw and low heat milks, cysteine was stimulatory while in high heated milks it was inhibitory. *Editor.*

In a previous paper, Greene and Jezeski *(9)* showed that the heat history of a NDMS sample exerted a measurable influence on the subsequent starter activity in that sample. Although this was among the first reports relating starter activity to the predrying treatment of powders, the phenomenon of microbial response to the heat history of culture media in general, and fluid milks in particular, has been extensively investigated. As early as 1890, Fokker *(5)* observed that heated goat's milk soured faster and showed a larger plate count after inoculation than did similar samples of raw milk. Since that time, the literature in this field has become voluminous and in many cases contradictory. Some investigators *(2, 4, 6, 13, 14)* reported that heating milk generally improved conditions for starter development. Other authors *(3, 7, 16)* indicated that the reverse was true. Furthermore, some reports cited treatments of from 75 to 80° C. for 30 minutes as optimal *(1, 15)*; whereas, others indicated that boiling for 2.5 hours *(4)* or heating to 115° C. for 15 minutes *(6)* resulted in the greatest activity. Tyler and Weiser *(17)* and Foster *(6)* compared starter activity in raw milk with that heated to 80° C. for ten minutes and that autoclaved at 115° C. for 15 minutes. Since starter activity improved in an orderly fashion with graded severity of heat treatment, these workers concluded that the improvement was due to a single mechanism which must be a function of heat intensity.

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[2] Taken from data submitted to the graduate faculty of the University of Minnesota by the senior author, in partial fulfillment of the requirements for the Ph.D. degree.

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From the practical point of view, the effect of heating milk on starter development is important in the manufacture of cheese and cultured milk. Most dairy plants employ a heat treatment before starter inoculation, to insure optimal development of the desirable bacteria as well as uniformity of the final product. Considering the discrepancies in the literature, it was decided to reinvestigate the effect of heat on milk and to attempt to resolve these contradictions. This study presents a summary of some investigations which compared the response of starters in milks with different heat histories. An attempt was also made to ascertain the reasons for the observed responses.

EXPERIMENTAL PROCEDURE

The raw fluid milks used in these trials were obtained from the University herd. Pooled milk, from healthy cows with no history of antibiotic therapy, was selected and delivered to the laboratory within four hours after milking. As soon as possible thereafter, the milks were heated in the following manner: The sample, stirred with a glass rod, was brought to the desired temperature in an Erlenmeyer flask immersed in boiling water. Depending on the temperature selected, this procedure lasted for from four to seven minutes. The flask was then covered with aluminum foil and transferred to a constant-temperature water-bath previously adjusted to the appropriate temperature. At the termination of the desired time exposure, samples were aseptically removed to sterile flasks and were cooled to 5° C. in less than five minutes. The samples were then dispensed and tempered for the starter activity tests *(9)*. Starter activity throughout this report refers to the titratable acidity produced by a standard inoculum after eight hours of incubation at 25° C. Any chemical or nutritional supplements (where used) were added to the tempered milks immediately prior to inoculation.

Casein supplements were prepared by suspending 50 gm. sodium caseinate (Difco) in 500 ml. water. After adjusting to pH 7.4 with a few drops of ten per cent lactic acid, the suspension was divided into two lots, each of which was subjected to the desired heat treatment. Serum protein was supplemented in the form of an exhaustively dialyzed, concentrated (4×) acid whey, which was sterilized by passing through a Selas filter. Both casein and whey were added at a rate designed to supplement the final milk with approximately seven gm. protein per liter. Cysteine was added from a stock solution of L-cysteine hydrochloride (Eastman Organic Chemicals). The stock solution was made up so that one ml. added to 100 ml. milk would result in a concentration of 1×10^{-7} moles/liter.

The increase in available −SH groups due to heat or addition of heated-dialyzed whey was estimated by the thiamine disulfide method of Harland and Ashworth *(11)*.

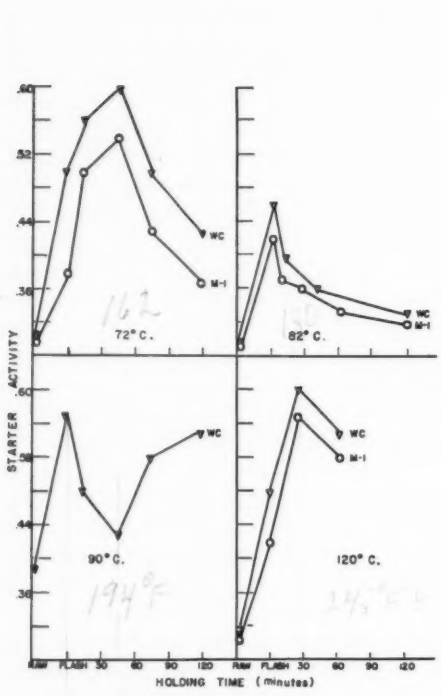
RESULTS

Typical response of starters when different time exposures at given temperatures are employed is shown (Figure 1). Flash-heating (less than 30 seconds)

at 72° C. improved the raw medium considerably, and further stimulation was observed after holding at this temperature for 15 and 45 minutes. A holding period of 75 minutes created inhibitory conditions for starter activity and the inhibition was intensified by the more severe treatment of 72° C. for two hours. A similar phenomenon of stimulation, followed by inhibition, was observed in milks heated to 82° C. for different time periods. In this case, however, the zone of stimulation was displaced to the shorter holding periods, and heat-induced inhibition was observed after relatively brief holding times. At 90° C., the previously observed stimulation was reproduced after momentary exposure, and the zone of inhibition occurred after 15 and 45 minutes. To this extent, heating at 90° C. corresponded qualitatively to heating at 72 and 82° C. Further heating, however, reimproved the milk as a substrate, and exposures of 75 minutes and two hours permitted the milk to approach the original optimal condition produced by flash-heating at 90° C.

The heat treatments at 120° C. introduced a new phase of starter responses to heat treatment. In this case, the initial stimulation-inhibition-stimulation cycle observed at the lower temperatures was masked, because of the difficulty of precisely timed sampling during early stages of heating in the autoclave; consequently, this appears on the chart as one zone of stimulation. The masking was due to the difficulty involved in exact control of heat exposure, especially during the first few minutes of heating, because of the relatively prolonged coming up and coming down times, which would contribute materially to the over-all heat exposure. Thus, the total effective heat treatment at this temperature for any given time exposure would be greater than the total effective treatment for a similar time exposure, under conditions where rates of heating and cooling could be carefully controlled. Consequently, the initial zone of stimulation observed at 120° C. probably encompassed the first three phases of stimulation and inhibition noted at lower temperatures. However, the inhibitory zone which resulted from extended autoclaving could not be related to the inhibitory zone previously shown at 72, 82, or 90° C., and may be considered as a fourth phase in a stimulation-inhibition cycle, occurring after the initial heat-induced inhibition had been overcome and the subsequent zone of stimulation terminated.

Because the cyclical nature of this relationship was previously unreported, further trials were performed to substantiate it. A variety of fluid milks, obtained and tested over a period of several months, gave results identical with those shown (Figure 1). Similarly, a variety of starters representing a wide spectrum of individual strains and mixtures of strains responded in the cyclical fashion described above. A typical trial showing the activity of ten different starters in milks subjected to a graded series of heat treatments is shown (Figure 2). A wide distribution of acidity values occurred in the raw milk. This was to be expected, considering the differences in nutritional and environmental requirements exhibited by each starter, as well as their varied sensitivity to any inhibitors that might be present in raw milk. In pasteurized milk, there was an improvement in activity by every starter tested, but here again a wide variation in individual acidities was noted, as well as a variation in extent of improvement



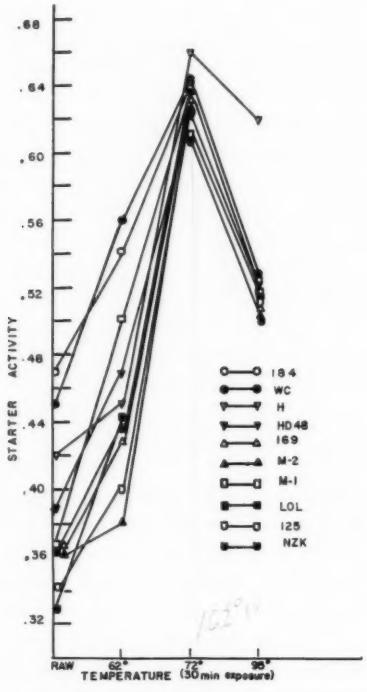


FIG. 1. The response of starters in milk exposed to constant temperatures for various holding periods. (One per cent inoculum; 8-hr. incubation at 25° C.)

FIG. 2. The response of ten different starters in one lot of milk exposed to different temperatures for a constant holding time. (One per cent inoculum; 8-hr. incubation at 25° C.)

attributable to pasteurization. Each starter showed an optimum response in milk heated at 72° C. for 30 minutes, and an inhibited activity as this exposure was exceeded. From the standpoint of practical dairy bacteriology, it is also noteworthy that the variation among the starters was at its minimum in milk heated to the optimal exposure.

Once it had been ascertained that there is a marked relationship between heat history and subsequent starter activity in a sample of milk, the next phase of these investigations dealt with the reasons for this relationship. Since the response of starters could be duplicated in either whole milk, skimmilk, or reconstituted NDMS, it was concluded that the responsible mechanism was associated with the nonfat fraction. Consequently, an investigation was made into the effects of heating on different milk proteins. A summary of this trial (shown Table 1) indicates that those temperatures which cause a change in milk for starter development have little or no effect on casein, but have a profound effect on the serum protein fraction. Furthermore, there is some evidence that both

TABLE 1

Response of two cultures propagated in variously heat-treated milks to the addition of heated and unheated casein and dialyzed whey (titratable acidity after 8 hr. at 25° C.)

Culture 1%	Heat treatment of milk	Casein		Dialyzed whey	
		Unheated	80° C.—10 min.	Unheated	80° C.—10 min.
M-1	Raw	0.330	0.340	0.290	0.305
	63° C.—30 min.	0.385	0.385	0.340	0.385
	80° C.—10 min.	0.475	0.475	0.515	0.455
WC	Raw	0.340	0.340	0.275	0.330
	63° C.—30 min.	0.410	0.420	0.380	0.500
	80° C.—10 min.	0.510	0.510	0.535	0.495

the stimulatory and inhibitory effects of heating, observed previously, could be related to the same chemical fraction of the milk. Thus, milk supplemented with casein behaved essentially the same, regardless of whether the casein was heated or unheated. On the other hand, the addition of milk serum proteins in the form of dialyzed whey had a marked influence on starter activity. Heated whey stimulated acid production in raw and low-heat milks; whereas, in high-heat milks it evoked a pronounced inhibition. This evidence is highly circumstantial, but it does provide a possible insight into the mechanism of heat-induced stimulation and inhibition.

An attempt was then made to discover why the serum proteins exerted this unique effect. These proteins are distinguishable from casein on the basis of their sulfur concentration and, more specifically, because they contain cysteine. Therefore, an experiment was performed in which starter response was assayed in a series of heated milks to which cysteine had been added. These data are plotted (Figure 3). In each case, the control milks behaved in the usual manner:

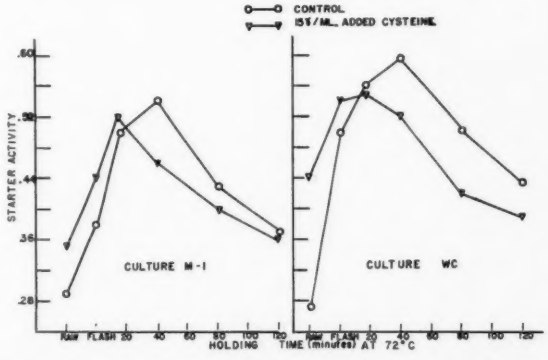


FIG. 3. The effect of added cysteine HCl on starter activity in milks subjected to a constant temperature for varying holding periods. (One per cent inoculum; 8-hr. incubation at 25° C.)

stimulation followed by inhibition, as the exposure was intensified or extended. Cysteine supplementation, on the other hand, had the same effect as the addition of heated serum protein: marked stimulation in the raw and low-heat milks, followed by definite inhibition in the highly heated samples. The exact exposure at which cysteine ceased its stimulation and became inhibitory corresponded to the time-temperature treatment required to denature 15 to 20% of the milk serum proteins *(12)*, and is in agreement with the denatured serum protein concentrations (from 0.15 to 0.20 mgm. N/ml) previously reported to be optimal for starter activity *(8)*.

These data, therefore, support the hypothesis that heat influences starter activity by its denaturing effect on the serum protein fraction of milk and, in particular, by making available either stimulatory or excessive concentrations of sulfhydryl groups. When cysteine is added to raw or low-heat milk samples, it is stimulatory. In highly heated samples, however, it supplements the cysteine already exposed, in much the same manner as if further heat were applied, and the ultimate total concentration of cysteine is inhibitory.

In an attempt to substantiate this hypothesis further, the responses of a starter culture were observed in a series of milks which had been altered by heating, cysteine addition, serum protein supplementation, and combinations of all three treatments. Determinations of thiamine disulfide–reducing substances (TDRS) were also made on these milks. The relationship of starter activity to TDRS is illustrated by the scattergram (Figure 4). Since the TDRS test is used as an estimate of heat-activated sulfhydryl groups, it is possible from these data

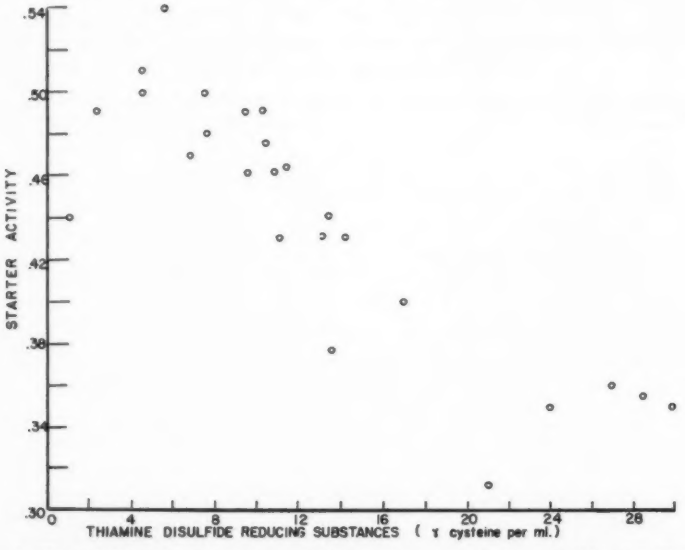


FIG. 4. The relationship of starter activity to the concentration of thiamine disulfide–reducing substances (TDRS) in milk. (One per cent inoculum; 8 hr. of incubation at 25° C.)

to establish a general relationship between cysteine concentration and starter activity. In particular, the chart illustrates the inhibitory effect of excess cysteine. Although there were too few samples of extremely high and low TDRS to warrant generalized conclusions, these data support the hypothesis that inhibition induced at heat treatments below 100° may be owing to an excess of available sulfhydryl groups.

DISCUSSION

It was found that, in general, heating raw milk improved its ability to support starter activity. On the other hand, a continued increase in heat treatment did not result in a consistent and uniform improvement in starter response. This response was not a simple linear function of heat treatment which could be extrapolated, but rather appeared to be cyclical in nature, with mild treatments such as 72° C. for 30 minutes stimulating starter activity, and more severe treatments evoking inhibition. Even more severe treatments (90° C. for 60 minutes) overcame this inhibition, and the most severe treatment studied, 120° C. for 60 minutes, introduced inhibition once more.

In practical dairy plant operations, the first three phases of this stimulation-inhibition-stimulation-inhibition cycle usually assume the greatest importance, namely those phenomana which occur at treatments below 100° C. Nonetheless, in those plants where milk for mother cultures is autoclaved, inhibition resulting from excessive heating at 120° must also be considered. However, in the latter case, sterility of the milk is the ultimate consideration, and choice of an exposure which could achieve this result and still not interfere with starter activity could be accomplished easily (see Figure 1). On the other hand, difficulties might arise in the case of the manufacturer who does not have facilities for heating under pressure and must choose an exposure below 100° to insure practical sterility and maximum starter activity. Data presented in this report might aid in the choice of the appropriate heat treatment. Similarly, information gained in this study might be of value to cottage cheese manufacturers, who must choose a heat treatment which results in satisfactory curd texture and body. Since the optimum exposure for starters (72° for 30 minutes) is considered too high for good cheese-making practice, a possible development might be the addition of a denatured serum protein source, or cysteine itself, to a low-heat milk, thus duplicating heat-induced stimulation without risking the deleterious effect of heat. A problem of this nature deserves further study.

The phenomenon of a heat-induced cycle of stimulation-inhibition-stimulation-inhibition might also explain certain apparent inconsistencies observed in the literature. Thus, the disagreement mentioned earlier, as to whether heat is stimulatory or inhibitory, can be reconciled in the light of this report. Essentially, it would be a question of the specific exposure a given investigator studied. Furthermore, inconsistencies regarding the different heat treatments suggested by different authors as optimal are not necessarily inconsistent. There actually are two different optima, one in the range of 72° for 30 minutes and the other in the range of 90° for two hours, with a suboptimal zone between these limits.

In essence, the data presented in this report do not contradict earlier reports by Foster *(6)* or Tyler and Weiser *(17)*. However, it can be shown that the series of exposures used by these authors would yield misleading results for, unless a larger number of heat treatments in the range of 60° to 100° C. were studied, the cyclical nature of heat-induced response would be overlooked. It is essential that this phenomenon be recognized in the formulation of a general hypothesis about stimulatory mechanisms.

A subsequent report *(10)* will attempt to define more closely the relationships between cysteine availability and starter activity here revealed. It should then be possible to outline a general hypothesis about the mechanism of starter response to heat-induced changes in milk.

SUMMARY

1. A study of starter responses in a series of fluid milks which had been subjected to heat treatments ranging from 62° C. for 30 minutes to 120° C. for 60 minutes revealed the following:
   (*a*) Gradual increases in severity of exposure resulted in an initial stimulation, followed by a zone of inhibition, followed by another zone of stimulation, and terminating in a second area of heat-induced inhibition.
   (*b*) The primary zone of stimulation occurred at exposures from 62° for 30 minutes to 72° for 40 minutes. The first zone of heat-induced inhibition was initiated at exposures equivalent to 72° for 45 minutes and extended through 82° for 10 minutes to 120 minutes, as well as at 90° from 1 to 45 minutes. This inhibition was arrested and stimulation was reinduced by heating at 90° for 60 to 180 minutes, or autoclaving (120°) for 15 to 30 minutes. The final zone of inhibition occurred after autoclaving for longer than 30 minutes.
2. There was a marked variation in starter responses in raw and pasteurized milk, but the differences between starters were considerably diminished in milks heated to 72 or 95° for 30 minutes.
3. The first and second phases of the heat-induced stimulation-inhibition-stimulation-inhibition cycle could be duplicated by the addition of denatured serum protein or cysteine hydrochloride. The transition from stimulation to inhibition occurred at exposures which resulted in concentrations of 0.15 to 0.20 mgm. denatured serum protein nitrogen or from 10 to 20 γ cysteine per ml.
4. Artificially supplemented cysteine augmented the sulfhydryl groups made available by heating. In raw and low-heat milks, cysteine was stimulatory; in highly heated milks, cysteine was inhibitory.

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STUDIES ON STARTER METABOLISM. III. STUDIES ON CYSTEINE-INDUCED STIMULATION AND INHIBITION OF STARTER CULTURES IN MILK [1, 2]

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The relationship of starter responses to available cysteine in milk subjected to various heat treatments was investigated. The stimulating effect of either heat or cysteine could be simulated by adding milk protein hydrolysate, expelling oxygen, and adding cystine, glutathione, or thioglycolate. The inhibitory effect of heat below 100° C. or excess cysteine was related to the amount of volatile sulfides in milk and could be simulated by bubbling hydrogen sulfide through milk. Initial stimulation appears to be due to lowering Eh, destroying heat-labile inhibitors, partially hydrolyzing protein, and denaturing serum protein. *Editor.*

Many attempts have been made to explain the mechanisms of heat-induced stimulation of starter activity in milk. One school of thought *(3, 4, 15, 17)* maintains that heat exerts its effect by inactivating native thermolabile inhibitors. Another suggests that starters respond because heating alters the oxidation-reduction potential *(7, 19)* and the nutritional characteristics of the medium *(8, 16)*. Even within this latter group there is disagreement. Foster *(8)* suggested casein hydrolysis as an improvement mechanism, Parker and Elliker *(16)* suggested a nonspecific increase in amino nitrogen, and Czulak and Meanwell *(5)* related improvement of activity after heating, to the destruction of a substance which rendered a growth factor unavailable. However, these attempts to explain the mechanism have been handicapped by an incomplete picture of the phenomena involved. The recent description by Greene and Jezeski *(10)* of a stimulation-inhibition-stimulation-inhibition starter activity cycle related to graded severity of heat treatments has reopened the question of mechanism(s) responsible for these phenomena. Furthermore, the implication by these authors of denatured serum protein and, more particularly cysteine, in both stimulation and inhibition of starter cultures might provide an approach to the solution of some aspects of the problem.

This report is a summary of several experiments designed to investigate more closely the relationship of starter response to available cysteine.

EXPERIMENTAL PROCEDURE

The basic experimental approach in this investigation involved attempts to duplicate by various means the effect of heating and/or of artificially added

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[2] Taken from data submitted to the graduate faculty of the University of Minnesota by the senior author, in partial fulfillment of the requirements for the Ph.D. degree.

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cysteine on starter activity. The starters used and the method of evaluating their response to different treatments are described elsewhere *(9)*. In this report starter activity, unless indicated otherwise, refers to the titratable acidity produced by a standard inoculum after eight hours of incubation at 25° C.

Chemical additives such as glutathione, cystine, and thioglycolate were prepared as stock solutions of appropriate concentration, so that one ml. added to 100 ml. of milk resulted in a final concentration of 10^{-7} moles/ml. Supplementary nitrogen was added to the milk, where indicated, from stock solutions of milk protein hydrolysate (BBL) so that one ml. of solution would provide the desired final concentration in 100 ml. milk. All supplements were added to the milk after it had been heated, dispensed, tempered to incubation temperature, and immediately prior to starter inoculation. In certain trials, the atmosphere during incubation was varied by bubbling nitrogen, hydrogen, or oxygen through the milk for 20 minutes before inoculation and throughout the incubation period. Several trials employed milks heated in an oxygen-free environment. These samples were processed in Erlenmeyer flasks fitted with rubber stoppers and glass tubing, arranged in such a fashion as to permit bubbling with nitrogen gas during heating and cooling. The milk treated in this manner was refrigerated under nitrogen until immediately before a trial and then was dispensed, tempered, and inoculated in the normal laboratory atmosphere.

Two techniques were adopted to measure the volatile sulfides generated by heating milk. The first method consisted of a visual comparison of the degree of blackening on a dried, lead acetate–soaked (one *N*) filter paper disk placed on the flask mouth during heating. The second method involved an iodimetric titration based on a technique used by Kallio and Porter *(13)*: the milks were heated, cooled, and tempered in the usual fashion, and 200 ml. were placed in a one-liter Erlenmeyer flask fitted with a two-holed rubber stopper. Nitrogen gas was bubbled through a glass tube immersed below the milk surface, and the escaping gases were passed through a train of two test tubes, each containing 10 ml. of normal alkaline cadmium acetate; the bubbling was carried on for one hour, after which the milk samples were acidified with five ml. of 6 *N* HCl and bubbling continued for another 30 minutes. At the end of this time the cadmium acetate solutions were pooled, acidified with 6 *N* HCl, and added to two ml. of 0.002 *N* I_2-KI in a 50-ml. Erlenmeyer flask. Using 0.5 ml. of a one per cent starch indicator, the iodine was titrated against freshly diluted 0.001 *N* sodium thiosulfate. The decrease in free iodine, as measured by the decrease in thiosulfate titer, was assumed to be proportional to the amount of volatile sulfide contained in the milk, and was converted by calculation to equivalents of H_2S. The nitrogen displacement of volatile sulfide was suggested by a technique used by Townley and Gould *(18)*.

RESULTS

In order to clarify the relationship between the response of starters in heated milk and their similar response to available cysteine, trials were performed in an attempt to duplicate the effect of cysteine by various means. The first aspect

to be investigated was the possibility that raw milk is nutritionally deficient, and that cysteine, added artificially or made available by serum protein denaturation, was necessary for adequate growth.

Results of typical trials which measured starter activity in milk supplemented with protein hydrolysate, compared to milk supplemented with cysteine, are presented (Figure 1). It appeared that the supplementary nitrogen (with

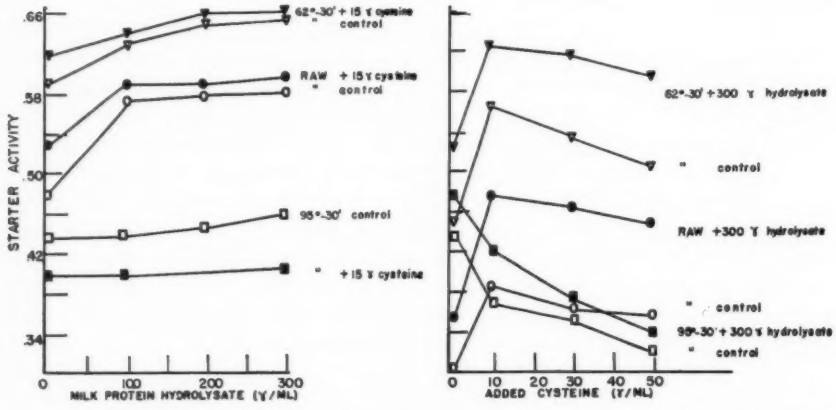

FIG. 1. Influence of a supplementary nitrogen source (milk protein hydrolysate) on the response of Culture WC to added cysteine HCl in milks with different heat treatments. (One per cent inoculum; eight hours incubation at 25° C.)

or without added cysteine) stimulated acid development in raw and pasteurized milk, but had a greatly reduced effect in the highly heated milk. This might signify a certain nutritional inadequacy that can be overcome either by heating or supplementing with cysteine. On the other hand, protein hydrolysate did not duplicate the inhibitory effect of cysteine in heated milk. Thus, it can be inferred from these data that, whereas cysteine-induced stimulation might be due in part to the overcoming of a nutritional deficiency, cysteine-induced inhibition in more highly heated milk is due to a different mechanism. Furthermore, the possibility that inhibition caused by cysteine or excessive heat might be due to tying up of essential nutrients is diminished by these data. Even when protein hydrolysate was added in excess, cysteine and heat exerted their inhibitory action.

Another attempt to define the possible mechanism of stimulation and inhibition by heat and/or by cysteine is shown (Table 1). Katznelson and Hood *(14)* showed that reducing substances were stimulatory in raw milk, because they overcame the inhibitory influence of dissolved oxygen. Results presented in this table support that contention. The starters grew well in a nitrogen atmosphere even in raw milk; whereas, they were markedly inhibited by oxygen even in heated milk. Thus, any lowering of oxygen tension, whether achieved by artificial cysteine addition or the exposure of reducing substances by heat-denatured

TABLE 1

Influence of different incubation atmospheres on the response of culture M-1 in milks with different heat treatments (titratable acidity after 12 hr. at 25° C.)

	Raw	62° C. — 30 min.	82° C. — 10 min.
Control	0.415	0.485	0.785
Oxygen	0.300	0.310	0.325
Hydrogen	0.540	0.750	0.760
Nitrogen	0.800	0.800	0.775

serum proteins, would tend to duplicate the effect of nitrogen-gassing and would stimulate starter activity. It can be seen, however, that nitrogen and hydrogen did not induce significant inhibition in the heated milk. Thus, while another mechanism of cysteine stimulation can be deduced, the inhibition phenomenon is still unclarified.

Further studies on the possible mechanism of cysteine action were made by testing the response of starter cultures to related chemical compounds. A comparison between cysteine, cystine, sodium thioglycolate, and glutathione is shown (Figure 2). All of the additives were stimulatory in raw and pasteurized

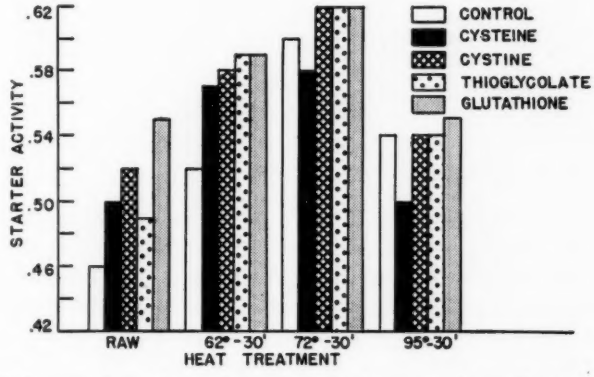

FIG. 2. Influence of cysteine and several related compounds on the starter activity of Culture WC in heated milks. (One per cent inoculum; eight hours incubation at 25° C.)

milk, but cysteine alone inhibited starter activity in milk with higher heat treatments. The improved response in raw milk to cystine and glutathione might be attributed to the nutritional requirements of the culture for protein-degradation products (see Figure 1). Similarly, the response to thioglycolate, and to some extent glutathione, might result from a reduced oxygen tension (see Table 1). However it is significant that the inhibitory influence of cysteine was unique, and that the toxicity induced by heated milk was sufficient to overcome the stimulation afforded either by reducing substances or extra nutrients.

Since some of the cysteine-induced stimulation apparently was due to a lowering of Eh, the possibility existed that the toxicity might also be the result

of a further drop in Eh. There was also the chance that inhibition might be due to some oxidation products which develop during atmospheric heating. Both of these conjectures were discarded upon review of the data (shown Figure 3). It is apparent that the inhibitory materials develop equally well in milks heated under nitrogen and under air. Furthermore, the former product (heated under

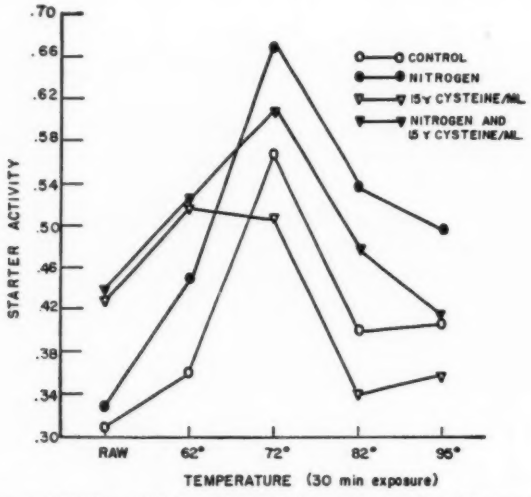

FIG. 3. Response of Culture M-1 in milks heated in air or under nitrogen and supplemented with cysteine HCl. (One per cent inoculum; 8 hours at 25° C.)

nitrogen), which should have a lower Eh than the latter (heated in air) *(11)*, was a better substrate after any heat treatment. The stimulation afforded by cysteine could in this case again be attributed to a lowering of Eh, since the effect could be duplicated but not enhanced by nitrogen. The toxicity, on the other hand, could neither be augmented nor overcome by nitrogen, suggesting a mechanism other than excessively low Eh or toxic oxidation products.

At this stage of the investigation, it was considered well established that cysteine duplicated the initial stimulatory effect of heating by lowering the Eh and/or by improving the nutritional character of the medium. The inhibition of starter activity, however, either by further heating or increasing the concentration of cysteine, or both, was still unexplained. This inhibition could not be overcome by nutritional supplementation, reducing agents, or heating under nitrogen; nor was it due to a suboptimal Eh. Furthermore, the inhibitory material was heat-labile as well as being heat-induced, since it was diminished by heating to exposures more intense than 90° for 60 minutes.

The first indication that volatile sulfides might be involved in this phenomenon was obtained from qualitative organoleptic observation. While heating the different milks used in these trials, it was evident that certain exposures evolved a characteristic "cooked" smell; whereas, more intense exposure treatments

yielded less pungent odors. A search of the literature on volatile sulfide evolution revealed that exposures required for maximum starter inhibition coincided with exposures required for maximum sulfide liberation *(18)*.

Consequently, a trial which measured volatile sulfide activity was performed in conjunction with a starter activity test. (For these results, see Figure 4.) A close inverse relationship existed between the degree of blackening of the lead acetate and the amount of acid subsequently produced by a starter culture

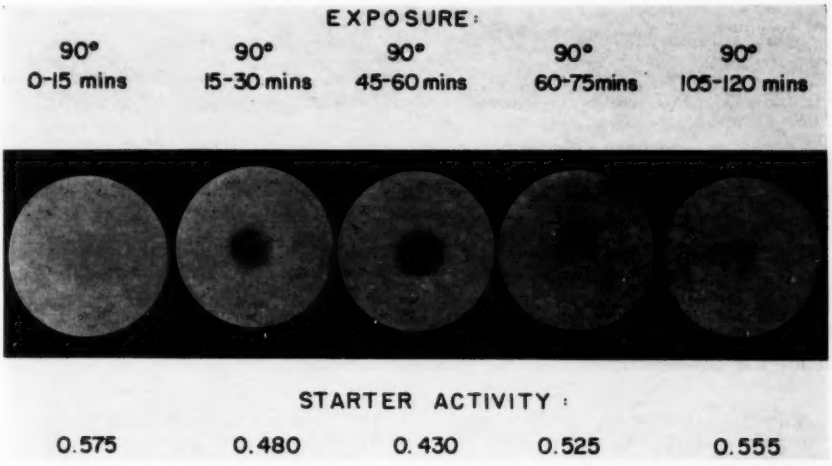

FIG. 4. The relative concentrations of sulfides liberated from milk during heating (measured by extent of darkening on lead acetate–soaked filter disks), and the response of Culture WC when grown in these milks.

in that milk. That sample which supported the poorest starter activity was the one in which the most sulfides had been evolved. Similarly, a diminution in sulfide liberation was reflected in improvement of starter activity.

Direct evidence that hydrogen sulfide bubbled through milk for a short time before inoculation was sufficiently toxic to inhibit starter activity is shown (Table 2). The inhibitory effect of the sulfide was sufficiently great to mask any other stimulatory or inhibitory properties of the substrate. This trial and the one illustrated in Figure 4 provided good circumstantial evidence than inhibition by heat and/or cysteine may result from toxic concentrations of volatile sulfides.

To substantiate this theory, it was necessary quantitatively, to measure the volatile sulfides in milk, to show that both heat treatment and addition of cysteine increased the titer of these sulfides and that the concentration of sulfides was related to starter activity. Evidence of this nature is presented (Figure 5). It can be seen that the maximum amount of sulfides was obtained from milk heated to 90° for 60 minutes, exposures above and below this resulting in a lower value. Furthermore, cysteine supplementation increased the concentration of

TABLE 2

Response of Culture WC in milk treated with H_2S gas prior to inoculation

	Starter activity	
	Control	H_2S[a]
Raw	0.295	0.235
Raw + cysteine (15γ/ml)	.545	.245
62° — 30 min.	.450	.240
62° — 30 min. + cysteine	.650	.230
72° — 30 min.	.620	.240
72° — 30 min. + cysteine	.610	.240
95° — 30 min.	.490	.255
95° — 30 min. + cysteine	0.430	0.250

[a] H_2S generated by heating mixture of sulfur, paraffin, and asbestos, and bubbling evolved gas into milk for ten seconds through a sintered-glass sparger.

measurable volatile sulfides. The activity of the starter was closely related to change in sulfide concentration in both normal and cysteine-supplemented milk.

DISCUSSION

The response of starter cultures to heat-induced alterations in their milk substrate is of practical and academic interest to dairy scientists. In a previous report *(9)*, it was shown that starter activity in reconstituted NDMS was closely related to the heat history of the powder. A subsequent paper *(10)* described

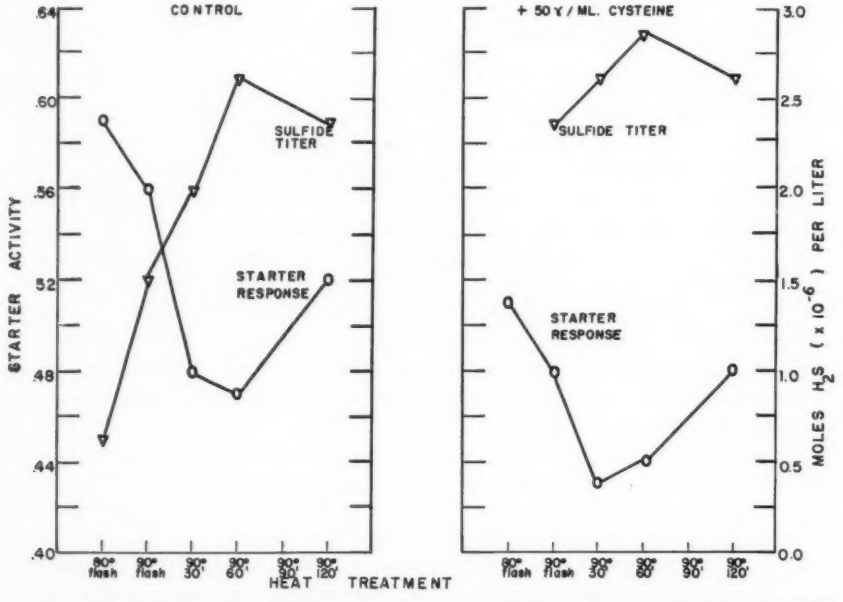

FIG. 5. Relationship between starter activity and volatile sulfide concentration. (Sulfides measured iodimetrically; one per cent inoculum; eight hours incubation at 25° C.)

both inhibitory and stimulatory effects which resulted during continued heat treatment of fluid milk. It was found that, to a certain extent, the influence of heat could be duplicated by the addition of denatured serum protein and cysteine.

On the basis of these previous findings, and the data presented in the current paper, it is possible to propose a general hypothesis that might explain the response of starters in heated milk.

The primary stimulation observed after heating raw milk to 62 and 72° C. for 30 minutes is probably due to a variety of individual mechanisms and their interaction. There is little doubt that one of these factors is the destruction of heat-labile inhibitors found in raw milk, probably the lactenins under investigation by Auclair and his coworkers *(1, 2, 3)*. However, this is not the only, nor necessarily the most important, inhibitor. The oxidation-reduction potential of agitated raw milk, which is unsatisfactory for acid production by starters *(6, 14)*, is also lowered by heating, due to the expulsion of oxygen and the exposure of reducing groups in denatured protein. This then becomes another factor to explain stimulation by mild heat (see Table 1). The possibility of nutritional improvement due to heat-induced protein hydrolysis is a third factor that should be considered (see Figure 1). However, the extent of protein hydrolysis which occurs below 80° C. is probably too limited to be of significance. A fourth factor which contributes to initial stimulation is the heat-denaturation of serum proteins, which is turn might render the protein more susceptible to proteolytic degradation, would contribute to a lowered Eh, and might play a role in lactenin inactivation *(20)*.

The combined involvement of all four of these mechanisms, namely lactenin inactivation, lowering of Eh, protein hydrolysis, and protein denaturation, is possible from both a theoretical and an experimental basis. The evidence presented in this paper shows that stimulation of starter activity by either heat or cysteine addition could be explained by any or all of the above-mentioned mechanisms. However, the zone of inhibition induced by exposures ranging from 72° for 30 minutes to 90° for 90 minutes, or the addition of excess cysteine, was a unique phenomenon unrelated to oxygen tension, milk-borne inhibitors, or nutrient level. It is proposed that this phenomenon results essentially from an excess of denatured serum protein and the concomitant excess of available −SH groups. Further evidence indicated that this mechanism was somehow related to the concentration of hydrogen sulfide and other heat-labile sulfides produced during the heating process. This hypothesis would also explain the second stimulation phase induced by heating above 90°. If the assumption is valid that starter inhibition is due to sulfide excess, improvement in starter activity should coincide with diminution in sulfide concentration. This was shown to be the case. Moreover, it could be shown that supplemented cysteine increased the volatile sulfide concentration in milk, thereby accentuating the inhibitory response already induced by heat.

The final zone of inhibition, resulting from extended autoclaving, was not studied, but it is suggested that mechanisms reviewed by Hartley *(12)* might be operative in this range.

SUMMARY

During a study of the response of lactic starters to heat-induced changes in milk, and the ability of added cysteine to duplicate some of these heat-induced changes, it was found that: *(1)* The stimulatory effect of either heat or cysteine could be duplicated by, nutritional supplementation with milk protein hydrolysate; expelling oxygen by nitrogen or hydrogen ebullition, and the addition of cystine, glutathione, or thioglycolate. *(2)* The inhibitory effect of excess heat (below 100° C.) or excess cysteine could be duplicated by ebullition with hydrogen sulfide, and was closely related to the concentration in milk of heat-evoked volatile sulfides.

The following hypothesis was advanced to explain the response of starters in heated milk:

(1) The initial stimulation is due to a variety of factors acting independently and in conjunction with each other. Among these factors are the lowering of Eh due to oxygen expulsion; the destruction of heat-labile inhibitors native to raw milk; the partial hydrolysis of protein, and the denaturing of serum protein.

(2) The first zone of inhibition is due to excess concentration of cysteine with the concomitant increase in toxic volatile sulfides.

(3) The second zone of stimulation is due to a heat-induced diminution in toxic sulfides.

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COMPARISON OF ANTIBIOTICS FOR DAIRY CALVES ON TWO LEVELS OF MILK FEEDING

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Growth studies were conducted with 96 Holstein calves to determine the effects of chlortetracycline, streptomycin, and a mixture of bacitracin and penicillin at three levels for two periods, and the effects of two levels of milk feeding. The antibiotics tended to increase the performance of the calves with greater effects on the calves fed the low milk ration. The mixture of bacitracin and penicillin was not as effective as the chlortetracycline and streptomycin except in controlling diarrhea. Calves fed 350 lb. of milk grew faster for the first seven weeks than those fed 175 lb., but the antibiotics minimized this by increasing the performance of the low-milk group. The antibiotics may have a milk-sparing effect. *Editor.*

The early reports of Loosli and Wallace *(11)*, Bartley *et al. (2)*, and Rusoff *(15)* stimulated considerable research on the value of antibiotics in the nutrition and upon the health of the dairy calf, and this has been reviewed by Knodt *(9)*, Reid *et al. (14)*, and Lassiter *(10)*. However, several papers pertinent to this study have appeared since these reviews. Swanson *et al. (19)* fed calves a liberal ration of milk and grain during early life and demonstrated no consistent increase in growth rate when chlortetracycline was added to the diet. The mean growth rates, however, were slightly in favor of the supplemented group. Bartley *et al. (3)* compared chlortetracycline with streptomycin and found streptomycin increased daily gain and feed efficiency and decreased scours somewhat, when compared with the control calves, but was not as effective as chlortetracycline in regard to any of these criteria. Both 30 and 90 mg. per 100 lb. body wt. per day of streptomycin were fed, whereas only the 30-mg. level of chlortetracycline was used. No differences were noted between the two streptomycin levels.

Research regarding the possibilities of antibiotics acting on calves through changes in digestibility of feed components is conflicting and incomplete. Bartley's results *(4)* did not indicate a consistent improvement in digestibility of any of the feed components by adding chlortetracycline to the ration of dairy calves at either nine or 13 weeks of age. Hogue *et al. (8)*, however, have obtained improvement in protein digestibility and nitrogen retention when chlortetracycline was added to the ration of calves. The same type of responses was obtained when bacitracin was used, but these responses were not as great as those observed with chlortetracycline. Radisson *et al. (12, 13)* have indicated that the effect of antibiotics may be through alteration of the intestinal flora of the dairy calf.

MATERIALS AND METHODS

The experiments reported herein were conducted during the spring, summer, and fall of 1953, and have been reported previously in brief form *(7)*.

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Ninety-six Holstein heifer calves (purchased at local auction) were used in two replicates of a 2×4×3×2 factorial experiment. The experimental variables included, (*1*) two levels of milk feeding: (*a*) 350 lb. of milk over a seven-week period and (*b*) 175 lb. over a five-week period; (*2*) four antibiotic treatments: (*a*) none (control), (*b*) chlortetracycline (Aureomycin), (*c*) bacitracin-penicillin (4:1 by weight), and (*d*) streptomycin; (*3*) three levels of antibiotic feeding: (*a*) 10 mg., (*b*) 20 mg., and (*c*) 40 mg. per 100 lb. body weight per day, and (*4*) two durations of antibiotic feeding: (*a*) seven weeks and (*b*) 16 weeks.

The antibiotics were fed to the calves twice daily, mixed with the milk during the milk-feeding period, and mixed with the concentrate following the milk-feeding period. Those calves receiving milk for only five weeks received the antibiotic in a small amount of water from the fifth to the seventh week. Good-quality mixed hay was fed ad libitum throughout the experiment. A calf starter was offered from the beginning of the experiment to a maximum of 4.0 lb. per calf per day. During the 13th week, the concentrate allowance was raised to 5.0 lb. per day by adding one lb. of a dairy ration (16% protein). At 14 weeks the calf starter was gradually replaced by the dairy ration. The calves were confined in individual tie stalls. They were turned regularly into a concrete yard for exercise. Iodized salt blocks were provided at all times.

Observations. The calves were weighed weekly. Body weights, as well as height at withers, and heart girth, were measured on three successive days: upon arrival, at seven and at 16 weeks of age. The means of these successive observations were used as the appropriate values in the analysis of the data. Milk, hay, and concentrate consumption was recorded daily. The relative feed efficiency was estimated as the calculated TDN consumed per lb. gain at both seven and 16 weeks. Milk was estimated to contain 16.3%, hay 50%, and concentrates 72% of TDN. The condition of the feces was observed daily and classified as to relative firmness. The total days of abnormal feces was then used as a measure of the incidence of diarrhea. Appearance ratings of the calves were made at seven and 16 weeks, using a score sheet as described by Slack *(17)*.

During the course of the experiment, four calves were lost from causes other than the experimental treatments (three from pneumonia and one from navel ill). Missing plot values were calculated in these instances *(6)*.

Data were analyzed statistically, using the analysis of variance technique *(18)* and the multiple range test of Duncan *(5)*. In the analyses at seven weeks, duration had not yet become a variable and the residual mean square after calculation of all interactions was used as the error term. At 16 weeks all interactions above the first order were pooled into the error term. The replicates were analyzed individually and all data were pooled for combined analyses.

RESULTS AND DISCUSSION

Composition of the concentrates and hays and the hay grades are given (Table 1). The average daily gains in weight and the increases in height at withers and heart girth are summarized (Table 2). Data relating to feed intakes, feed efficiency, and the incidence of abnormal feces are also shown (Table 3).

TABLE 1

Chemical composition of the feeds and federal hay grades of the hay

	Chemical composition					
	Moisture	Protein	Ether extract	Crude fiber	Ash	N.F.E.
	(%)					
Replicate I						
Hay[a]	8.44	16.69	1.84	29.75	5.93	37.35
Calf starter[c]	11.68	22.37	2.38	6.53	6.01	51.03
16% dairy ration[d]	11.55	16.36	3.20	6.94	7.02	54.93
Replicate II						
Hay[b]	7.52	16.80	1.56	27.56	5.51	41.05
Calf starter[c]	7.60	23.81	2.58	6.98	7.20	51.83
16% dairy ration[d]	7.27	17.50	3.66	9.11	4.90	57.56

[a] U. S. Sample Grade alfalfa heavy grass mixed hay (slightly moldy).

[b] U. S. Sample Grade leafy alfalfa light timothy mixed hay (slightly moldy).

[c] Contained, in pounds: wheat bran 200, crushed oats 350, cracked corn 355.5, linseed oil meal 200, alfalfa meal 140, corn distillers solubles 100, irradiated yeast 0.5, corn and cane sugar molasses 160, soybean oil meal 360, dried skimmilk 100, ground limestone 10, dicalcium phosphate 10, iodized salt 10, vitamin A feeding oil 4; and 2 gm. cobalt sulfate.

[d] Contained, in pounds: corn gluten feed 160, wheat bran 220, wheat standard middlings 100, hominy feed and cornmeal 380, ground oats 440, soybean oil meal 200, rice bran 80, corn distillers dried grains 160, cane and corn sugar molasses 220, dicalcium phosphate 15, ground limestone 5, iodized salt 20; and 2 gm. of cobalt sulfate.

TABLE 2

Effect of antibiotics and two levels of milk-feeding on the growth rates of dairy calves

		Average daily gain				Increase in height at withers		Increase in heart girth	
Age (wk.)		0-7	0-7	0-7	0-16	0-7	0-16	0-7	0-16
Replicate		I	II[e]	I & II	I & II	I & II	I & II	I & II	I & II
Lb. milk	Obs/ rep	(lb.)				(in.)			
				Control					
350	6	0.80	1.00	0.90	1.16	2.5	5.1	4.3	10.4
175	6	0.71	0.62	0.67	1.06	1.9	4.7	3.2	9.5
Av.	12	0.75[b]	0.81[b]	0.78[b]	1.11	2.2[b]	4.9	3.8[b]	10.0
				Chlortetracycline					
350	6	1.04	1.04	1.04	1.22	2.8	5.2	4.8	11.0
175	6	1.00	0.98	0.99	1.18	2.4	5.1	4.7	10.4
Av.	12	1.02[a]	1.01[a]	1.01[a]	1.20	2.6[a]	5.1	4.8[a]	10.7
				Bacitracin-Penicillin					
350	6	1.02	1.04	1.03	1.17	3.0	5.4	4.8	10.4
175	6	0.84	0.73	0.78	1.03	2.1	4.6	4.2	9.8
Av.	12	0.93[a]	0.88	0.91[a]	1.10	2.5[a]	5.0	4.5[a]	10.1
				Streptomycin					
350	6	1.00	0.91	0.96	1.19	2.5	5.1	4.7	10.6
175	6	0.89	0.88	0.88	1.18	2.5	5.1	4.8	10.4
Av.	12	0.95[a]	0.89	0.92[a]	1.19	2.5[a]	5.1	4.7[a]	10.5
Av. 350	24	0.96	1.00[c]	0.98[c]	1.19[c]	2.7[c]	5.2	4.6[c]	10.6[c]
Av. 175	24	0.86	0.80[d]	0.83[d]	1.11[d]	2.2[d]	4.9	4.2[d]	10.0[d]
S.d. (obs.)		0.21	0.16	0.19	0.17	0.69	0.88	1.00	1.25

[a] > [b] ($P < 0.05$).

[c] > [d] ($P < 0.05$).

[e] Antibiotic treatment × milk-level interaction significant ($P < 0.05$).

TABLE 3

Effect of antibiotics and two levels of milk-feeding on incidence of diarrhea, concentrate and hay consumption, and feed efficiency of dairy calves

		Av. days abnormal feces			Concentrate consumption		Hay consumption		TDN consumed per lb. gain	
Age (wk.)		0-7	0-7	0-7	0-7	0-16	0-7	0-16	0-7	0-16
Replicate		I	II	I & II	I & II[c]	I & II	I & II	I & II	I & II	I & II
Lb. milk	Obs/ rep				*(lb.)*					
					Control					
350	6	7.3	3.0	5.2	35.9	290.1	18.2	161.7	2.28	2.76
175	6	6.3	2.3	4.3	45.2	301.6	23.5	145.8	2.40	2.77
Av.	12	6.8[a]	2.7	4.8[a]	40.5	295.8	20.8	153.8	2.34[a]	2.76[a]
					Chlortetracycline					
350	6	2.7	2.0	2.3	30.0	272.2	22.1	168.4	1.99	2.55
175	6	1.5	3.0	2.2	63.6	310.8	24.9	175.1	1.91	2.65
Av.	12	2.1[b]	2.5	2.3[b]	46.6	291.5	23.5	171.7	1.95[c]	2.60[b]
					Bacitracin-Penicillin					
350	6	2.5	2.5	2.5	33.4	266.8	24.1	172.6	1.96	2.66
175	6	1.8	0.8	1.8	41.8	270.4	32.8	169.4	2.15	2.75
Av.	12	2.2[b]	1.0	2.1[b]	37.6	268.6	28.5	171.0	2.06[b]	2.71
					Streptomycin					
350	6	0.8	5.0	2.9	30.7	270.3	21.9	169.2	2.04	2.59
175	6	2.0	0.8	1.4	59.4	321.5	23.0	161.4	2.08	2.65
Av.	12	1.4[b]	2.9	2.2[b]	45.0	295.9	22.4	165.3	2.06[b]	2.62[b]
Av. 350	24	3.3	3.1	3.2	32.5[b]	274.8[b]	21.6	168.0	2.07	2.64
Av. 175	24	2.9	2.0	2.4	52.4[a]	301.0[a]	26.1	162.9	2.13	2.71
S.d. (obs.)		4.2	2.5	3.4	17.7	46.4	12.2	44.6	0.29	0.24

[a] > [b] ($P < 0.05$).
[b] > [c] ($P < 0.05$).
[c] Antibiotic treatment × milk-level interaction significant ($P < 0.05$).

Effect of antibiotic supplementation. Data presented indicate that antibiotic supplementation had a desirable effect on the growth and well-being of young dairy calves during the early weeks of life. All of the antibiotics employed in this experiment significantly ($P < 0.05$) increased average daily gain, resulted in a greater increase of heart girth and height at withers, improved feed efficiency, and decreased the days of abnormal feces for the first seven weeks. At the end of sixteen weeks the effect of antibiotics was expressed only by an increase in feed efficiency. As can be seen (Table 2), the antibiotics demonstrated their effects on diarrhea in the first replicate only. The calves showed little scouring in the second replicate. It should be noted that the antibiotics were never effective in eliminating scours but only in decreasing its incidence.

Further examination of the data suggests that a ranking of the antibiotics can be made according to their relative effect on the criteria measured. The only criterion where the bacitracin-penicillin mixture was clearly as effective as either chlortetracycline or streptomycin was its effect in decreasing incidence of diarrhea. Chlortetracycline and streptomycin showed comparable effects on height at withers, heart girth, and feed consumption, but chlortetracycline was

somewhat more effective in increasing average daily gain and feed efficiency, and both were superior to the bacitracin-penicillin mixture.

When calves were fed 350 lb. of milk, all antibiotics tested gave approximately the same responses. However, several interactions were noted between antibiotic treatment and milk level. Calves fed only 175 lb. of milk showed greatest responses to chlortetracycline, with streptomycin following closely. Both were superior to the bacitracin-penicillin mixture. This general milk × antibiotic interaction was noted for most observations, but reached significant proportions only in the case of average daily gain for the second replicate and concentrate consumption for both replicates at seven weeks. Apparently, the more effective antibiotics have a milk-sparing action and allow lower milk-feeding regimes to be successfully employed without impairment of growth or health of the calves.

Effects of level of milk feeding. The level of milk feeding had a definite effect on the growth and consumption of hay and grain by the calves up to seven weeks. Most of the differences present at seven weeks were not as important at 16 weeks (Tables 2 and 3). The occurrence of several interactions between antibiotics and milk level was also observed and has been discussed. It is evident from this study that feeding 175 lb. of whole milk without an effective antibiotic will not result in as satisfactory a performance as 350 lb. (Tables 2 and 3).

Effects of level and duration of antibiotic feeding. These experiments indicate that there were no differences of importance due either to level or duration of antibiotic feeding. The average daily gains to seven weeks of all calves receiving 10, 20, and 40 mg. of antibiotics were 0.94, 0.97, and 0.94 lb., respectively. It appears from these data that 10 mg. was as effective as 40 mg., but considering past research *(1, 16, 20)* one is reluctant to assume that 10 mg. would be as effective as 40 mg. under all conditions. The average daily gain to 16 weeks of age for all calves fed antibiotics during seven weeks was 1.14 lb., as compared with 1.18 lb. for those fed antibiotics for the entire 16-week period. The absence of a difference indicates that antibiotics are most effective for dairy calves during the early weeks of life.

Other observations. Some information as to the mode of action of the antibiotics was obtained from further analysis of the data. An adjustment of average daily gain data, for the influence of abnormal feces by analysis of covariance, had no appreciable effect on the relative significance of the antibiotic treatments, suggesting that improvement in rate of gain as a result of antibiotic supplementation is not entirely a result of the reduction in scours. This effect can also be noted in the data presented (Tables 2 and 3). While the bacitracin-penicillin mixture and streptomycin were as effective as chlortetracycline in decreasing scours, they were not as effective in increasing the daily gains to seven weeks. Similarly, the data from the second replicate indicate an increase in gains on the 175-lb. milk level, owing to antibiotic supplementation, even though digestive disturbances were few. An increase in gain in the absence of severe diarrhea was not observed when calves were fed a more liberal amount of milk (Tables 2 and 3). These data suggest that calves receiving a high-milk diet will not benefit from antibiotic feeding if intestinal disorders are minor.

Hay-consumption measurements were made, but were so variable that few important differences were observed. The hay fed in the second replicate was apparently of better quality than that fed in the first and, as a result, more was consumed. Total pounds of hay consumed per calf were 18.9 and 28.8 and 131.0 and 199.9 at seven and 16 weeks, respectively. It is interesting that when the average estimated net-energy intakes of the calves were calculated at 16 weeks, the two replicates were essentially the same: *viz.*, 270 therms for Replicate I and 277 therms for Replicate II. The calves consuming the less desirable hay had compensated by consuming more starter. No differences were detected in any of the appearance-rating data and thus they are not included.

SUMMARY

Growth studies with 96 Holstein heifer calves were conducted to determine the effects of chlortetracycline, streptomycin, and a mixture of bacitracin and penicillin at three levels and two durations of supplementation, and the effects of two levels of milk feeding.

A general increase of growth rate, decrease in incidence of digestive disturbances, as well as increases in concentrate consumption and feed efficiency, were observed as a result of antibiotic supplementation. The antibiotics chlortetracycline and streptomycin were especially effective in increasing the performance of the calves on the lower milk-feeding schedule. Although the mixture of bacitracin and penicillin improved most criteria over the control calves, it was equal to chlortetracycline and streptomycin only as it affected incidence of diarrhea. Chloretetracycline was slightly more effective than was streptomycin in increasing feed efficiency and average daily gain to seven weeks.

The level of milk feeding had a definite effect on the growth of the calves. Calves fed 350 lb. of milk grew faster for the first seven weeks than those fed 175 lb. of milk. In most criteria observed, the antibiotics tended to minimize this difference by increasing the performance of the lower milk-fed group. The antibiotics were effective on the 350-lb. milk group only in the presence of digestive disorders.

Feeding antibiotics at a level of 10 mg. per cwt. live weight daily was as effective as either 20 mg. or 40 mg., and feeding for seven weeks was as effective as a 16-week feeding period.

It is suggested that the effects of the antibiotics on incidence of diarrhea and average daily gain are, at least in part, independent and that the antibiotics may have a milk-sparing effect in allowing the use of lower milk-feeding regimes.

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EFFECTS OF TEMPERATURE AND TIME OF INCUBATING AND PH OF PLATING MEDIUM ON ENUMERATING HEAT-TREATED PSYCHROPHILIC BACTERIA[1]

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A study was made to determine the effects of time and temperature of incubation and of the pH of the medium on heated cultures of P. fluorescens, P. fragi, and P. putrefaciens. The heated psychrophiles did not grow as well during the early phase of incubation as did the unheated ones, and their lag was greater at 5, 32, and 35° C. than it was at 25° C. The heated psychrophiles were more sensitive to pH changes than their unheated controls. *Editor.*

In recent years, numerous reports have appeared on different aspects of psychrophilic bacteria. Very little information, however, is available on the biochemical characteristics of psychrophiles which have been subjected to a sublethal treatment with heat, chemicals, or radiation. It has been shown *(3, 5, 8)* that the plating medium has a pronounced effect on the number of survivors of bacterial cultures subjected to a sublethal treatment with heat or radiation.

Nelson *(5)* reported that certain heat-treated bacteria are more exacting in their nutritional requirements than normal cells. Recently, Heather and Vanderzant *(3)* showed that a greater number of heat-treated survivors of *P. fluorescens* were found on complex media, such as nutrient agar and yeast extract agar, than on simple synthetic media. The addition of glutamic acid, proline, histidine, glycine, and methionine allowed heat-treated cells to grow in greater numbers on the simple synthetic media.

Lawton and Nelson *(4)* reported that surviving heat-treated psychrophiles had greatly increased lag phase in milk at 5 or 10° C., but at 25° C. only a slight increase was observed. During the early phase of growth a greater number of surviving heat-treated psychrophiles were found on standard plate count agar than on tryptone glucose extract (TGE) agar.

Nelson *(6)* has shown that the pH of the plating medium most favorable for growth of surviving heat-treated bacteria was not the same as for normal cells.

This study was initiated to determine the effects of time and temperature of incubation and of the pH of the medium on survivors of heat-treated cultures of *P. fluorescens*, *P. fragi*, and *P. putrefaciens*.

EXPERIMENTAL METHODS

Five strains each of *P. fluorescens*, *P. fragi*, and *P. putrefaciens* were used as test organisms. These organisms were from the stock culture collection of

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the Department of Dairy Science at Texas A & M College. They were identified by reference to Breed *et al. (2)*. The stock cultures were carried in litmus milk and were transferred weekly. For the individual experiments, the cultures were grown in sterile skimmilk for 24 hr. at 25° C.

In the heating trials, one ml. of a 24-hr. milk culture was added to 99 ml. of sterile skimmilk which had been adjusted to the desired temperature in a constant-temperature water-bath. The suspension was agitated continuously by a stirrer. At certain intervals samples were taken and cooled immediately in sterile screw-cap test tubes submerged in ice water. The levels of population in the nonheated cultures were adjusted to those of the heated cultures, by diluting them with sterile skimmilk. The rate of dilution was based upon schedules previously established for each culture. In no instance was the number of survivors greater than 0.1%. Aliquots of the heat-treated and nonheated suspensions were plated on tryptone glucose yeast extract agar *(1)*. The plates were incubated at 35 and 32° C. for two days, 25° C. for two and three days, 15° C. for three, five, and seven days, and at 5° C. for five, seven, and ten days. Duplicate plates were made of each dilution, for each combination of time and temperature of plate incubation.

In the experiments on the effect of pH of the plating medium on the number of surviving heat-treated psychrophiles, the plates were incubated at 25° C. for three days. Aliquots of heat-treated and nonheated suspensions were plated on tryptone glucose yeast extract agar *(1)* adjusted to pH values ranging from pH 5.0 to 10.0.

RESULTS

Effect of time and temperature of plate incubation on bacterial counts. Data on the effect of plate incubation time on the count at different plate incubation temperatures are presented (Table 1). Both heated and nonheated (control) bacterial suspensions were used. The count at the shorter plate incubation period(s) was expressed as per cent of the count obtained after maximum plate incubation time. In most instances, lower counts were found when the incubation time was shortened. The data also indicate that the heated cells did not express themselves as readily on the plates during the early part of the incubation period as did the nonheated cells. This lag was more pronounced at the lower plate incubation temperature. When the plates were incubated at 25° C. for 2 days, the count of each of the nonheated cultures was at least 75 per cent of that observed after the maximum incubation period (three days). However, under similar conditions of plate incubation, this was true for only eight of the 15 heated cultures. After incubating the plates for seven days at 5° C., the counts of 11 of the 15 cultures were at least 75% of those observed after ten days of incubation. However, the count of each heat-treated culture after seven days at 5° C. was less than 75% of that obtained after incubation of the plates for ten days at 5° C. In general, the data indicated that heated cells did not develop as rapidly on the plates as did nonheated cells.

TABLE 1

Effect of incubation time on counts at different temperatures of heat-treated and nonheated psychrophiles

Treatment		Heated[a]					Control				
Incub. temp. (° C.)		25	15		5		25	15		5	
Max. incubation time (days)		3	7		10			7		10	
Incubation time (days)		2[b]	3[b]	5[b]	5[b]	7[b]	2[b]	3[b]	5[b]	5[b]	7[b]
P. fluorescens	2	98	62	97	0	60	95	86	93	95	98
	3	34	8	56	0	0	91	96	97	14	97
	70	25	48	65	0	0	104	33	26	32	109
	E	92	29	43	0	16	105	80	89	45	79
	L11	10	8	20	44	45	109	70	90	80	88
P. fragi	1	70	52	92	<1	6	91	95	100	92	102
	2	87	49	64	0	21	93	98	101	83	91
	2M	102	26	94	0	4	90	100	100	84	85
	13	81	0	44	0	0	92	104	114	0	97
	L13	72	54	90	<1	2	101	94	100	24	96
P. putrefaciens	3	82	57	58	0	29	76	79	80	0	60
	5	52	0	12	0	0	85	14	86	0	6
	10	90	53	80	0	0	75	79	91	0	19
	11	62	67	90	0	4	98	84	98	12	82
	13	82	2	78	0	<1	80	71	97	0	5

[a] The cultures received the following heat treatment: *P. fluorescens* 2, 3, 70, E, and L11—5.5 min., 2.5 min., 1.5 min., 45 sec., and 1.5 min., respectively, at 55° C. *P. fragi*—45 sec. at 60° C. *P. putrefaciens*—1 min. at 50° C.

[b] The count at the shorter incubation time is expressed as per cent of the count obtained after maximum plate incubation time.

Data on the influence of plate incubation temperature on the count after maximum incubation time are presented (Table 2). The counts obtained at the different plate incubation temperatures were expressed as per cent of the count observed at 25° C. The count obtained after incubating the plates for three days at 25° C. was selected as 100, because previous work *(7, 9)* indicates that temperatures of from 20 to 25° C. are optimum for growth of many psychrophiles.

The data (Table 2) indicate that the counts of both heated and nonheated cultures at 35° C. were very low compared with those obtained when the plates were incubated at 25° C. At 32° C., nine of the 15 nonheated cultures had counts comparable to those observed at 25° C. Only one of the heated cultures, however, had at 32° C. a count comparable to the one obtained at 25° C. In general, the counts of both heated and nonheated cultures at 15° C. were very similar to those obtained at 25° C. The counts of 12 of the 15 nonheated cultures at 5° C. were at least 75% of those observed at 25° C. Only three of the 15 heated cultures showed counts at 5° C. comparable to those obtained at 25° C. In general, the data indicate that plate incubation temperatures of 35, 32, and 5° C. are less suitable for the enumeration of heat-treated psychrophiles than are 25 or 15° C.

Effect of pH of plating medium on bacterial counts. Data on the effect of the pH of the plating medium on the count of heated and nonheated psychrophiles are presented (Figures 1, 2, and 3). Maximum counts of *P. fluorescens, P. fragi,* and *P. putrefaciens,* after a sublethal heat treatment in skimmilk, were obtained with the plating medium adjusted to pH 6.0 to 8.0, 6.0 to 8.0, and 7.0, respectively.

TABLE 2

Comparison of counts[a] at different incubation temperatures of heat-treated and nonheated psychrophiles

Treatment		Heated[b]					Control				
Incubation temp. (° C.)		35	32	25	15	5	35	32	25	15	5
Incubation time (days)		2	2	3	7	10	2	2	3	7	10
P. fluorescens	2	2	52	100	102	46	<1	99	100	99	99
	3	0	0	100	90	10	23	97	100	103	95
	70	0	7	100	128	0	0	74	100	74	67
	E	0	15	100	90	11	0	97	100	119	104
	L13	<1	24	100	106	40	2	106	100	108	79
P. fragi	1	<1	22	100	110	93	<1	77	100	100	100
	2	<1	26	100	94	8	<1	102	100	101	102
	2M	0	18	100	114	19	0	99	100	103	110
	13	7	19	100	90	5	<1	99	100	106	106
	L13	0	26	100	132	15	0	94	100	100	81
P. putrefaciens	3	0	64	100	120	110	0	50	100	90	72
	5	<1	<1	100	95	<1	<1	70	100	90	7
	10	0	99	100	74	58	0	106	100	97	85
	11	0	30	100	104	36	0	46	100	100	86
	13	0	8	100	129	122	0	44	100	107	79

[a] The counts at 35, 32, 15, and 5° C. are expressed as per cent of the count obtained at 25° C.
[b] See footnote[a], Table 1.

Maximum counts of the nonheated controls were obtained at pH values ranging from 6.0 to 8.0. With the heated cultures of *P. fluorescens* and *P. fragi*, a rapid decrease from the maximum count was observed at pH values above 7.0. None of the cultures showed growth on the plates at pH 5.0 and 10.0. Unheated cultures of these species gave essentially the same count over a wide range of pH (from 6.0 to 9.0). In most instances growth was observed at pH 10.0. All nonheated cultures of *P. fragi* showed growth at pH 5.0.

The nonheated cultures of *P. putrefaciens* showed similar counts at pH values of 7.0 and 8.0. The cultures subjected to a sublethal treatment with heat were very sensitive to the pH of the plating medium. The majority of the cultures did not show growth on plates at pH values below 7.0. A rapid decrease in count was observed at pH values above 7.0. In most cases no growth was observed at pH 5.0, 6.0, 9.0, and 10.0. The nonheated cultures of *P. putrefaciens*, however, were much less sensitive to the pH of the plating medium than were the heated cultures.

In general, the data indicate that psychrophiles subjected to a sublethal heat treatment are more sensitive to the pH of the plating medium than the nonheated controls.

DISCUSSION

Results of the present study with nonheated psychrophiles indicate that incubation for periods shorter than three days at 25° C., seven days at 15° C., and ten days at 5° C. usually gave lower counts than at maximum plate incubation time. Incubation of the plates at 25° C. for three days seemed preferable for the enumeration of the nonheated psychrophiles. The majority of the cultures did not show growth on the plates at 35° C. In some cases incubation at 32 or 5° C. resulted in lower counts than at either 25 or 15° C. In general, these findings are

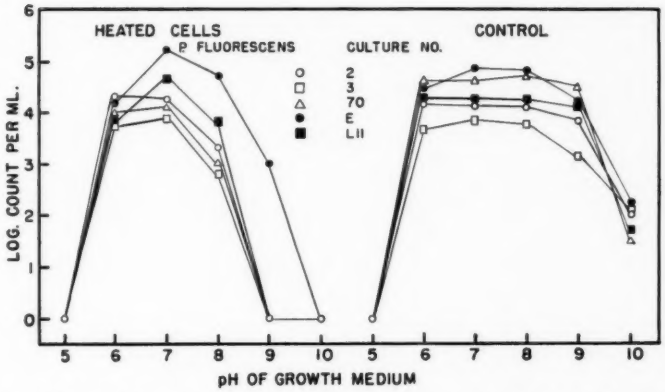

FIG. 1. Effect of the pH of the plating medium on the counts of heated and nonheated cultures of *P. fluorescens*. (Heat treatment, Cultures 2, 3, 70, E, and L11—5.5 min., 2.5 min., 1.5 min., 45 sec., and 1.5 min., respectively, at 55° C.)

in agreement with those reported by Nelson and Baker *(7)* and Vanderzant and Moore *(9)*.

The results with surviving heat-treated psychrophiles indicate that incubation of the plates at 25 or 15° C. usually gave maximum counts. In most cases the counts at 32 and 5° C. were much lower than those obtained at either 25 or 15° C. Most heat-treated cultures did not show colony formation on the plates at 35° C. These results indicate that a plate incubation temperature of 35 or 32° C., as recommended by *Standard Methods (1)*, probably will result in too low a count if part of the flora is psychrophilic in nature. Incubation at 5° C. for seven days, as recommended *(1)* for the enumeration of psychrophiles, would give similar results. Results of the present investigation, and the work of Nelson and Baker *(7)*

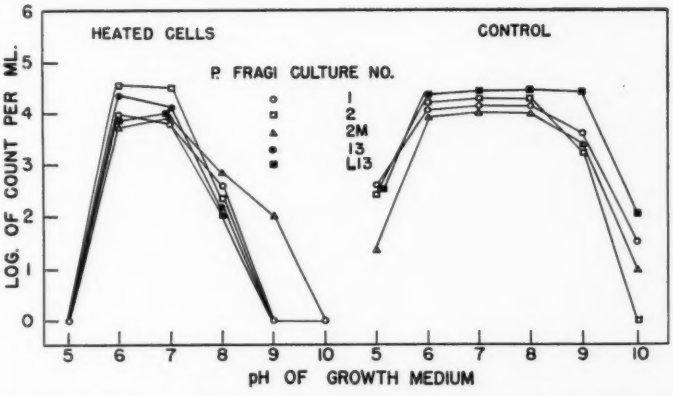

FIG. 2. Effect of the pH of the plating medium on the counts of heated and nonheated cultures of *P. fragi*. (Heat treatment, 45 sec. at 60° C.)

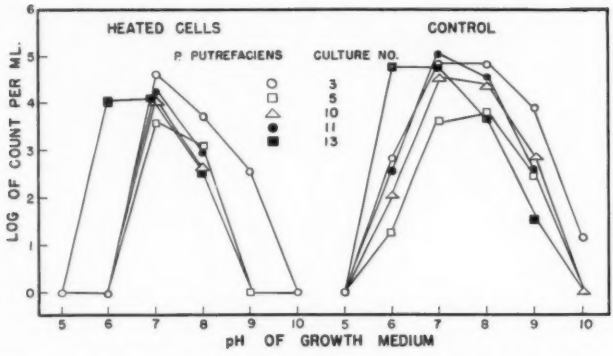

FIG. 3. Effect of the pH of the plating medium on the counts of heated and nonheated cultures of *P. putrefaciens*. (Heat treatment, 1 min. at 50° C.)

and Vanderzant and Moore *(9)*, indicate that incubation of the plates for three days at 25° C., or seven days at 15° C., usually would result in a maximum count of both heated and nonheated psychrophiles. Incubation at 25° C. seems preferable over that at 15° C., since the counts are available sooner and less equipment is involved.

Surviving heat-treated psychrophiles did not grow as readily on the plates during the early phase of incubation as did the nonheated cells. This was especially true at 5° C. Furthermore, the counts of heated cultures at either 5, 32, or 35° C. were much lower than those obtained at 25 or 15° C. (Table 2). These findings seem to indicate that from 15 to 25° C. is favorable for the development of heat-treated psychrophiles. The possibility exists that from 15 to 25° C. is favorable for some repair-mechanism to take place. One might argue that optimum conditions for growth exist at from 15 to 25° C. and that the conditions for resumption of growth are more favorable at these temperatures than at 5° C. However, with the counts expressed as per cent of the count obtained at 25° C., those of the heated cultures were in most cases much lower than those of the nonheated cultures. This indicates that some factor other than growth rate is responsible for this phenomenon. Lawton and Nelson *(4)* studied the resumption of growth of heat-treated psychrophiles in sterile skimmilk. They also observed an increase lag phase at 5° C.

Data on the influence of the pH of the plating medium on the count indicate that nonheated cultures of *P. fluorescens* and *P. fragi* gave essentially the same count over a wide range of pH values (from 6.0 to 9.0). Maximum counts of heat-treated cultures were obtained over a much more narrow pH range. Essentially the same results were reported by Nelson *(6)* for some other types of bacteria.

Data on the influence of the pH of the plating medium on the count of the test cultures of *P. putrefaciens* indicate the importance of the pH of the medium in the enumeration of certain heat-treated psychrophiles. This is clearly indi-

cated, for example, by *P. putrefaciens* Culture No. 3 (heated) at pH 7.0 and 6.0. At pH 7.0, 28,000 cells per ml. were found; at pH 6.0, none.

It is generally accepted that the psychrophiles commonly found in dairy products are destroyed by pasteurization. Therefore, they can be considered as postpasteurization contaminants from equipment, utensils, and faulty practices. Sanitizing equipment and utensils by heat is a common practice in dairy plants. This treatment with steam or hot water does not always result in complete sterilization. Some of the survivors, their number depending on the extent of the exposure to heat, may contaminate fluid dairy products and grow if the conditions are favorable. In enumerating these heat-treated cells, we should keep in mind that they seem to require somewhat different conditions for resumption of growth than do normal nonheated cells.

Very little information is available on the influence of chemical sanitizers on the biochemical characteristics of bacteria, psychrophiles in particular. Lawton and Nelson *(4)* reported that a sublethal treatment with chlorine did not modify the growth characteristics of certain psychrophiles.

If bacterial counts are desired of fluid dairy products which were held under refrigeration for long periods, it seems desirable (*a*) to incubate the plates for three days at 25° C., and (*b*) to adjust carefully the pH of the plating medium to 7.0. This procedure most likely will result in a maximum recovery of the flora which can be expected in such a product.

SUMMARY

Results of this investigation indicate that (*a*) surviving heat-treated psychrophiles did not grow as readily on plates during the early phase of the incubation period as did the nonheated controls, (*b*) the lag in their development on the plates was more pronounced at the high (35, 32° C.) and the low (5° C.) incubation temperatures than at 25° C., and (*c*) surviving heat-treated psychrophiles were much more sensitive to the pH of the plating medium than the nonheated controls.

ACKNOWLEDGMENT

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A COMPARISON BETWEEN THE SERUM INORGANIC IODINE LEVELS OF FEEDING POTASSIUM AND CUPROUS IODIDE, AND COPPER RETENTION

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The oral administration of cuprous and potassium iodide to calves for seven days did not result in significant differences in blood serum inorganic iodine levels. The elimination rate of iodine for both compounds was the same, being a linear function of the log serum inorganic iodine level with time. There was no evidence of toxicity from either iodine or from copper. Cuprous iodide was more palatable than potassium iodide. *Editor.*

Experiments by Long *et al. (8)* have shown that feeding 2.816 g. potassium iodide per day, administered at two-day intervals, is sufficient to maintain a high therapeutic serum inorganic iodine level constantly, for the systemic treatment for actinomycosis and other mycotic infections in cattle. Lewis *(7)* showed that very high levels of serum inorganic iodine could be obtained by feeding one gram cuprous iodide to a thyroidectomized Jersey bull. Mittler and Benham *(9)* found that cuprous iodide has greater efficacy than potassium iodide in protecting the thyroid gland of albino rats from enlargement. They attributed this effect to the insolubility of cuprous iodide contributing to slow absorption and a more continuous supply of iodine.

Cuprous iodide is more stable, it has no effect on vitamins B and C, and it yields a homogenous mixture more readily than does potassium iodide *(1)*. These advantages warranted further investigation of the value of cuprous iodide for the treatment of mycotic infections in livestock.

The purpose of this study was twofold: namely, to determine and compare the serum inorganic iodine levels of potassium iodide and cuprous iodide under a dosage regimen for the systemic treatment of mycotic infections in cattle; and to determine copper retention resulting from cuprous iodide administration.

EXPERIMENTAL PROCEDURE

Four Angus calves weighing 480 $\pm$ 10 lb., from purebred registered stock selected from the same farm, were paired into two groups. After one week of acclimatization, blood samples were taken twice from each calf before iodide administration, in order to determine basal serum inorganic iodine levels. The dosage regimen consisted of the oral administration via hard gelatin capsules containing either potassium iodide or cuprous iodide. Each group of calves was assigned and given one of the two compounds at 24-hour intervals for seven consecutive days. In the case of the group getting potassium iodide, 1.5 g. was given each calf. In the other group, the same amount of iodine derived from

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cuprous iodide was given as for the potassium iodide group. During the entire dosage regimen, blood samples were taken in the morning prior to dosage, or about 24 hours after the previous dose. Blood samples were drawn on the first, second, third, and ninth post-administration days, in order to follow the rate of elimination of inorganic iodine.

The serum inorganic iodine levels were determined by the method of Connor *et al. (3)*. Statistical analysis showed the data for the standard curve to depart significantly ($P<0.05$) from linearity, when absorbance was expressed as a function of the log of the concentration, in the range of 0.02 to 0.50 γ iodine per cc. The best over-all functional form, from the point of view of both convenience and precision, was found to be a second-degree polynomial, fitted to the data by the method of least squares, expressing concentration over a range of 0.06 to 0.50 γ iodine per cc. as a function of the transmittance. In this range of concentration, the maximum error between the average of triplicates and the six-point fitted curve was 4.4%. Serum inorganic copper levels and the copper content of liver were determined by the method of Peterson and Bollier *(10)*. A Beckman DU spectrophotometer and Corex cells having a one-cm. light path were used in the colorimetric procedures. Duplicate samples were run on the same serum sample for iodine values, in triplicate for copper values in the cuprous iodide–fed group, and in quadruplicate for copper values in the potassium iodide–fed group. The analysts did not know the group to which a compound had been assigned, and those administering the compounds did not know the results obtained by the analysts.

RESULTS AND DISCUSSION

Preadministration serum inorganic iodine levels. The data resulting from serum inorganic iodine levels determined on the samples taken four days before, and on the morning just prior to, initial dosage were each analyzed as an incomplete two-factor analysis, one factor with replication *(2)*. A significance level of 0.05 was used in this and subsequent analyses. Iodine levels within and between animal groupings four days before iodide administration were considered homogeneous within the precision of the assay method. The grand mean was 1.3 γ %, the mean square assay within samples variation being 0.5002 with four d.f. Results of the analysis on samples taken just prior to iodine administration, although indicating significant differences between calves within groups, evidenced no significant difference between groups. The grand mean was 3.5 γ %, the mean square assay within samples variation being 0.0892 with four d.f. The difference between grand means may reflect a true increase in serum inorganic iodine levels which accompanied the weaning process that occurred during the week of acclimatization. In order to obtain the values on the samples taken four days before iodine administration, it was necessary to go below the range of 0.06 γ iodine per cc., where the analytical errors were on the order of 10 to 20%, a fact which for the most part probably accounts for the difference in precision

between days. In presenting and interpreting the results of the administration and post-administration data, the individual calf means of the iodine levels on the morning just prior to dosage are subtracted from those of their corresponding succeeding levels.

Administration serum inorganic iodine levels. During the iodine-fed days, the levels derived from cuprous iodide invariably are higher than the corresponding potassium iodide levels (Figure 1); however, by examining the data

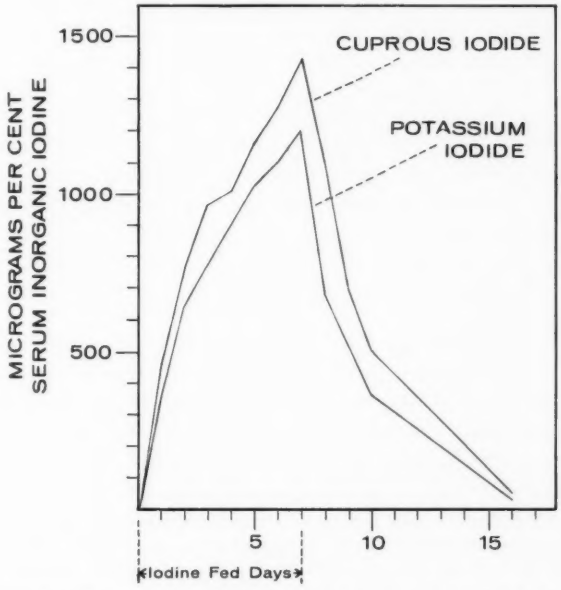

FIG. 1. Comparison between potassium iodide and cuprous iodide, fed at the same iodine level, on the serum inorganic iodine levels of calves.

as a trebly incomplete four-factor analysis, with one factor having a triplicate order of replication *(2)*, and using the results as a guide, it appears that within the sensitivity of the test no significant difference between compounds has been demonstrated. The sensitivity of the experiment was such that had the cuprous iodide levels been on the order of 150 γ % greater, a statistically significant result would have been obtained. Lack of sensitivity resulted from a very large difference in response between calves within compounds. As a result, a large number of animals would have to be tested before statistically significant results could be obtained. The difference in levels between days within compounds is more significant than 0.001.

Post-administration serum inorganic iodine levels. The data were analyzed as indicated for the iodide-fed days; it appears no significant difference in the rate of elimination of iodine derived from the compounds and accumulated

in the inorganic serum fraction over the seven-day dosage regimen has been demonstrated (Figure 1). The rate of iodine elimination was more precipitant than that observed for potassium iodide by Long *et al. (8)*, a difference which for the most part can be described as a characteristic response between different age-group animals, such as observed by Hansard *et al. (5)*. At the end of 24 hours after the last dose, the average serum inorganic iodine level for all calves had dropped to about 66% of the highest levels, at 48 hours to 46%, at 96 hours to 32%, and at 216 hours to two per cent. The rate of iodine elimination is exponential, as noted by Keating and Albert *(6)*. That the linear relation of the log of serum inorganic iodine level as a function of time can be used to represent approximately the rate of elimination, is shown by the fact that the ninth day post-administration level based on the first three such levels predicted a value of four per cent; whereas, the observed level was two per cent.

Thus, it appears that in very young animals, where iodism is being induced to treat mycotic infections, oral feeding of potassium iodide or cuprous iodide should be administered no less often than at one-day intervals, to maintain a high level of blood inorganic iodine. Alternatively, if it were desired to maintain, say, a level of 500 γ % in calves, it might be more practical to give 1.15 g. of iodine derived from potassium iodide, or cuprous iodide, for one day, followed daily by one-half this amount.

Copper serum level in the blood. Data resulting from copper serum levels at the seventh-day peak iodine levels were analyzed as an incomplete two-factor analysis, one factor with replication *(2)*. The mean copper serum level for the potassium iodide group was 0.904 γ per cc.; that for the cuprous iodide group, 1.15 γ per cc., and the mean square assay within-calves veriation was 0.02758 with ten d.f. The blood from the pair of calves fed cuprous iodide retained both its normal color and coagulation time. No significant difference in copper serum levels between the groups was demonstrated. It is likely that the cuprous iodide group does, in fact, reflect somewhat higher copper serum levels than does the potassium iodide group, but the fact is obscured by lack of precision in the assay method. If the mean copper serum level for the cuprous iodide group is taken as a basis for comparison with that of the potassium iodide group, the higher values does not indicate a toxic level, for, as observed by Davis *(4)*, cattle showing signs of copper toxicity have blood copper values 300 to 400% higher than those of normal animals.

Copper retention in the liver. Another group of cattle in a weight range of 750 lb. was fed 1.02 g. copper derived from cuprous iodide for 27 days, followed by an eight-day normal diet before sacrifice. The concentration of copper in the livers on a wet basis was as follows: Control Animal 1, 65 p.p.m.; Test Animal 2, two different samples, 120 and 147 p.p.m.; Test Animal 3, 206 p.p.m. These data indicate that although copper is deposited in the liver at the highest dosage level, and for twice the treatment time used in veterinary practice to obtain the concurrent high serum inorganic iodine levels for systemic acute mycotic therapy, the amount of copper deposited cannot be considered toxic. Davis *(4)* has found that in a group of cattle consuming

copper sulfate at a rate such that a 500-lb. animal obtains 1.25 g. elemental copper per day, at least 50% of the animals will develop copper toxicity in from three to four months; further, the copper content of the livers of such animals is usually over 1,600 p.p.m., and sometimes as high as 3,500 p.p.m.

No evidence of toxicity from iodism or hyperthyroidism was noted in any of the test animals, nor were signs of copper toxicity or other adverse effects noted in the cuprous iodide–fed groups. In the 27-day feeding period cuprous iodide was observed to be very much more palatable to cattle than potassium iodide, a fact of much importance in the veterinary therapeutic application.

SUMMARY AND CONCLUSIONS

When 1.15 g. of iodine, derived in one case from potassium iodide and in the other from cuprous iodide, was administered orally to calves each day for seven days, cuprous iodide maintained the higher serum inorganic iodine level throughout the experiment; however, the data are not sufficient to show that the difference is statistically significant.

A serum inorganic iodine level of at least 500 γ% can be maintained in calves by the oral feeding of 1.15 g. of iodine, derived either from potassium iodide or from cuprous iodide at one-day intervals.

The rate of elimination of iodine, derived from potassium iodide and cuprous iodide, and picked up in the inorganic iodine serum fraction over the seven-day dosage regimen, was judged equivalent. The rate of iodine elimination can be defined approximately as a linear function of the log serum inorganic iodine level upon time, over the nine-day post-administration interval.

Serum copper levels for the cuprous iodide–fed group are likely somewhat higher than those for the potassium iodide group at the seven-day peak iodine level; however, the serum copper level is well within the range of that for normal animals.

The livers of calves on a cuprous iodide regimen, furnishing iodine at the maximum levels currently used in the systemic treatment of mycotic infections, and for twice the usual treatment time, were found to give copper concentrations from 2 to 5 times below those of cattle evidencing copper toxicity.

No evidence of toxicity from iodism or hyperthyroidism was noted in any of the test animals, nor were signs of copper toxicity or other adverse effects noted in the cuprous iodide–fed group.

Cuprous iodide, in addition to showing greater stability as a compound and greater compatability with feed ingredients, was found to be much more palatable to cattle than potassium iodide.

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FACTORS INFLUENCING METABOLIC ACTIVITY OF BULL SPERMATOZOA. I. 37, 21, AND 5° C.

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The aerobic metabolic activity of spermatozoa was compared in isotonic phosphate and saline diluents at 37, 21, and 5° C. Fructolysis decreased more at 37 to 21° C. than it did at 21 to 5° C.; whereas, oxygen consumption dropped more rapidly at lower temperatures. Marked differences between nine per cent sodium chloride and phosphate diluents at 37° C. were not observed at either 21 or 5° C. *Editor.*

The effective suppression of the motility of mammalian spermatozoa by decreasing their temperature to 5° C. and lower, and the general reversibility of that suppression, have made temperature control the method of choice in most practical attempts to prolong the functional life of mammalian spermatozoa.

It has been assumed that accompanying the effects of temperature on motility there is a parallel suppression and reversal of spermatozoan metabolic activity. Evidence for this assumption is based largely on observations with semen of the ram *(6, 7, 9, 10)*, and under experimental conditions in which only a part of the potential metabolic activity of the sperm cells could be expressed.

Recently, it has become increasingly clear that bull spermatozoa exhibit a wide metabolic potential, depending upon the conditions imposed in their study. Under aerobic conditions, which may occur with some of the techniques employed in artificial insemination practice *(14)*, and in the female reproductive tract *(3)*, bull spermatozoa may utilize a variety of metabolic substrates, and their metabolic activity may include the formation *(19)*, as well as the degradation, of fructose.

Bishop and Salisbury *(4)* have shown that phosphate ions depressed the motility and respiration of bull semen at body temperature in comparison with that occurring in 0.9% NaCl. This depression in oxygen uptake was accompanied by an accumulation of lactic acid, resulting in an aerobic catabolic pattern of carbohydrate utilization similar to that of anaerobiosis *(13)*. As phosphate ions have been a common constituent of many diluents used for both metabolic studies and practical storage of bull semen, the question arose as to whether or not the aerobic metabolic pattern at lower than body temperature is influenced in the same manner by phosphate.

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Recent investigations involving the development of media for room-temperature storage of bull semen *(17)* have emphasized the need for more information on the metabolic activity of spermatozoa at or near that temperature.

The experiments herein reported were designed to compare the aerobic metabolic activity of bull spermatozoa in phophate-containing and saline diluents at 37, 21, and 5° C., simulating body, room, and the normal, above-freezing, storage temperatures. From such comparative data, the ratio of the velocity constant for a given metabolic activity at one temperature to the velocity constant at a temperature 10 C. lower, Q_{10}, could be calculated.

MATERIALS AND METHODS

Bull semen of good initial motility was used and in most cases the ejaculates contained at least 200 million spermatozoa per ml. after dilution. The semen was diluted at room temperature (25° C.) at the rate of one volume of semen to four volumes of diluent, as soon after collection as possible (usually within two hours). However, in the last experiment the semen was cooled to 5° C. before dilution.

The diluents used were *(a)* 0.9% NaCl and *(b)* a solution isosmotic with *(a)*, prepared by mixing 0.13 *M* $Na_2HPO_4 \cdot 12\ H_2O$ and 0.17 *M* $NaH_2PO_4 \cdot H_2O$ to a pH of 7.0.

In the initial experiments on the effects of the temperature of incubation, the diluted semen was cooled slowly to 5° C., at the rate of about a one-degree drop in two minutes. One-ml. aliquots of semen were pipetted into cooled (5° C.) Warburg flasks, which were transferred to the Warburg bath. Two baths were used for each experiment, one operating at either 5 or 21° C. and the other at 37° C. The periods of incubation were four hours (37° C.) and eight hours (5 and 21° C.); longer periods were not possible, because of the rather poor survival of semen in this series. Motility estimates of the rate and percentage of motile spermatozoa were made at the start and finish of all experiments. The rate was scored on a scale ranging from 0 to 4 *(4)*.

Aliquots of semen were taken at the beginning and at the end of tests, for estimations of fructose or reducing substances and lactic acid. In the first series, the semen was frozen and stored before chemical determinations were made, but in all the other trials it was deproteinized immediately and the filtrate frozen. Lactic acid was determined by the method of Barker and Summerson *(1)*; fructose by Mann's *(7)* modification of the method of Roe *(12)*, and total reducing substances by a method using the chromogenic mixture of Nelson *(11)* and the reducing agent of Somogyi *(15)*. The initial and final pH were determined with a Beckman glass electrode pH meter. The uptake of oxygen was measured by the direct Warburg technique using air as the gas phase *(16)*. Except for the initial experiments on temperature effects, where one-half of the flasks were not shaken, the shaking rate was 116 c.p.m. with a stroke of 3 cm. The Q_{10} values were calculated from the following:

$$\text{Log } Q_{10} = \frac{10(\text{Log } k_1 - \text{Log } k_2)}{t_1 - t_2},$$

where k_1 = velocity constant at the higher temperature, t_1; k_2, that for the lower temperature, t_2.

All the results were tested by the analysis of variance, or "t" test, and the significant F, or "t", values are given in the text, with the appropriate degrees of freedom as subscripts.

The aerobic metabolism of bull semen was studied in four separate series of experiments. In the first, an incubation temperature of 5° C. was compared with 37° C., and in the second series 21° C. was compared with 37° C., under otherwise identical conditions.

Using slightly different conditions, 21° C. was later compared with 37° C. in a series of tests in which the effects of cold-shock also were being studied, and in the fourth group the effects of 5, 21, and 37° C. were examined concurrently.

In the first two experiments, the semen was diluted prior to cooling, with either 0.9% NaCl or the isosmotic phosphate buffer. Because of the necessity for slow cooling to prevent cold-shock, a period of about two hours elapsed between dilution and the start of the experiment. One-half of the flasks used were shaken at 116 c.p.m. and the remainder left stationary for the experimental period.

RESULTS AND DISCUSSION

Because the spermatozoa were actively motile at 37° C. in the diluents, and thus maintained the fluid contents of each flask in constant agitation, no significant effects of shaking were found in the first two experiments. Results are combined for presentation (Table 1). The two groups of data at 37° were also combined, and analysis of variance showed that the uptake of oxygen was significantly higher in NaCl than in phosphate [$F_{(1, 12)} = 38.8$, $P. < 0.01$], but that both lactic acid and fructose changes were greater in phosphate [$F_{(1, 12)} = 17.2$, $P<0.01$ and $F_{(1, 12)} = 5.6$, $P<0.05$, respectively].

Analysis of the 5 and 21° C. data showed that under the conditions of these tests the NaCl and phosphate diluents did not differ significantly in their effects on metabolism.

TABLE 1

Summary of the data from the initial tests of the effects of temperature on the aerobic metabolism of unwashed bull semen

		Aerobic Z[a] values of semen			+Z LA / −Z Fruct.
Temperature	Diluent	−Z O_2	−Z Fruct.	+Z Lactic	(×100)
(°C.)		*(μl.)*	*(μg.)*	*(μg.)*	*(%)*
5	NaCl	0.2	3.2	5.2	162
5	$HPO_4^=$	0.3	6.4	3.4	53
21	NaCl	0.7	2.7	1.3	48
21	$HPO_4^=$	0.7	0.9	0.9	100
37	NaCl	4.5	12.7	9.9	78
37	$HPO_4^=$	2.7	21.1	17.8	84

[a] Z = Activity/10^8 cells/hr.

In the third experiment, the semen was diluted at room temperature and metabolic measurements begun within one-half hour of dilution. Results are given (Table 2), analysis of which showed that there were no significant

TABLE 2

Effects of incubation at 21 and 37° C. on the aerobic metabolism of unwashed bull semen (means of seven ejaculates)

Temperature	Diluent	Aerobic Z[a] values of semen			+Z LA / −Z Fruct.
		−Z O_2	−Z Fruct.	+Z Lactic	(× 100)
(°C.)		(μl.)	(μg.)	(μg.)	(%)
21	NaCl	1.2	0.5	−0.5	—
21	$HPO_4^=$	1.1	5.1	1.5	30
37	NaCl	8.6	19.7	14.5	74
37	$HPO_4^=$	4.4	32.9	23.9	73

[a]Z = Activity 10^8 cells/hr.

differences between the NaCl and phosphate diluents at 21° C. for any of the metabolic characters, but at 37° C. the oxygen uptake was higher in NaCl than in phosphate [$t_{(6)} = 5.62$, $P < 0.01$].

In the fourth series of trials, in which the effects of 5, 21, and 37° C. were studied concurrently, the effect of 20% egg yolk in the NaCl and phosphate buffers was also examined. For this test, all semen was cooled to 5° C. before dilution. Both lactic acid and reducing-sugar changes were determined, but respiration was not measured.

The results for lactic acid, reducing sugars, and the final motility scores are summarized (Table 3). Analyses of variance for the metabolic data were made, but because the variances were not homogeneous, separate analyses were done for each temperature. At 5° C. there were no significant main effects, but at 21 and 37° C. the accumulation of lactic acid was significantly higher in phosphate than in NaCl. Egg yolk did not significantly influence lactic acid accumulation at any temperature, but at 37° C. the utilization of carbohydrate was increased by the presence of egg yolk, possibly due to more rapid and preferential utilization of the glucose in the egg medium *(18)* over the fructose provided in the semen.

At the end of the experiments, the motility of spermatozoa in 0.9% NaCl was consistently higher than in phosphate at each temperature. This superiority of the 0.9% NaCl occurred consistently, in spite of its mean final pH of 5.75, as compared to that of 6.54 in the phosphate diluent. Motility at the lower temperatures was improved by egg yolk. However, yolk did not improve livability in either the saline or phosphate at the highest temperature.

It was considered that the conditions under which the four groups of experiments were conducted were sufficiently similar to warrant the calculation of approximate Q_{10} values from the pooled data. Accordingly, Q_{10} for the ranges of 37 to 21° C. and 37 to 5° C. were calculated for fructose utiliza-

TABLE 3

Summary of the data from the second series of tests, of the effects of temperature on the aerobic metabolism of unwashed bull semen (five ejaculates)

Temperature	Diluent	Motility	Aerobic Z[a] values of semen		+Z LA	
			−Z Red. subst.	+Z Lactic Acid	−Z Red. subst. (× 100)	
(°C.)		(motility % × rate)	(μg.)	(μg.)	(%)	
5	NaCl	118	0.9	−2.2	—	
5	NaCl + yolk	175	4.7	2.4	51	
5	$HPO_4^=$	70	7.4	2.5	34	
5	$HPO_4^=$ + yolk	159	3.5	4.1	117	
21	NaCl	122	0.8	2.1	263	
21	NaCl + yolk	158	8.2	1.5	18	
21	$HPO_4^=$	55	12.1	10.7	88	
21	$HPO_4^=$ + yolk	120	30.1	11.5	34	
37	NaCl	130	41.9	30.2	72	
37	NaCl + yolk	131	62.4	34.5	55	
37	$HPO_4^=$	27	59.5	45.3	76	
37	$HPO_4^=$ + yolk	25	76.4	70.4	92	

[a]Z = Activity/10^8 cells/hr.

TABLE 4

Temperature coefficients (Q_{10}) of spermatozoan aerobic metabolic activity

Metabolite	Temperature ranges	
	(37–21° C.)	(37–5° C.)
Fructose	4.0 (17)[a]	1.6 (11)
Lactic Acid	4.1 (17)	2.2 (6)
Oxygen	2.8 (12)	4.2 (7)

[a]Figures in parentheses are the numbers of individual semen samples involved in each mean shown.

tion, lactic acid accumulation, and oxygen consumption, and are shown (Table 4).

These results indicate that the rate of fructolysis falls off more rapidly between 37 and 21° C. than between 21 and 5° C. On the other hand, the more limited data on the oxygen consumption of bull semen indicate the reverse situation.

The data of Mann *(7)* for undiluted ram semen show a gradual fall in fructolysis as the temperature was lowered to 24° C. Below 24° C., the decline was very rapid. A similar trend was found by Moore and Mayer *(9)* also, for the change in reducing-sugar, although for lactic acid accumulation the rate of decline remained constant over the range of 37 to 11° C.

A temperature of 5° C. has been used widely for the storage of suitably diluted semen for short periods of a few days. In view of the considerable decrease in metabolism at 21° C., as compared with 37° C., temperatures in the range of 5 to 21° C. also may be found useful, particularly if combined with other means of conserving the vital function of spermatozoa.

SUMMARY

The fructose utilization, lactic acid accumulation, and oxygen consumption of unwashed bull spermatozoa diluted with 0.9% NaCl or phosphate diluents were determined at 37, 21, and 5° C. The rate of aerobic fructolysis decreased more in the temperature range of 37 to 21° C. than it did from 21 to 5° C.; whereas, oxygen consumption appeared to fall more rapidly in the lower temperature range. The marked differences shown by the 0.9% NaCl and the phosphate-containing diluent at 37° C. were not observed at either 21 or 5° C. Temperature coefficients (Q_{10}) for spermatozoan aerobic metabolic activity are presented.

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FACTORS INFLUENCING METABOLIC ACTIVITY OF BULL SPERMATOZOA. II. COLD-SHOCK AND ITS PREVENTION

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Evidence is presented to show the effect of cold-shock on the permeability of the sperm cell, as revealed by the mineral content of the seminal plasma, the metabolic activity of the semen, and the protection by lecithin and egg yolk. Rapidly cooling diluted semen from 25° to near 0°C. greatly reduced motility, respiratory activity, and glycolysis. Metabolic damage was retarded by lecithin and almost completely by egg yolk, which also stimulated glycolysis of unshocked semen. Cold-shocking semen transferred potassium and ether alcohol–extractable phosphorus from cells to plasma, with an uptake of sodium and calcium by the cells. *Editor.*

When spermatozoa from several mammalian species are cooled rapidly from 25 to 5° C., or lower, without freezing, they undergo an apparently irreversible loss of motility which has been called temperature- or cold-shock by Milovanov *(18)*. More recently, the term thermal-shock has been adopted by Smith *(22)*, but the earlier term, cold-shock, will be used in this discussion, for there is evidence that sudden increases in temperature are not deleterious *(26)*.

The occurrence and prevention of cold-shock has been discussed by Birillo and Puhaljskii *(1)*, and Gladcinova *(10)*, who were able to prevent it by slow-cooling semen from body temperature to 0° C. for from three to four hours. Easley *et al. (8)* and others *(26)* have reported similar findings and, after Phillips and Lardy *(20)* had recommended the use of egg yolk as a protective agent for the storage and transport of semen, found that cold-shock did not occur in the presence of egg yolk.

Attempts to isolate the active protective factor in egg yolk have been made by Mayer and Lasley *(17)*, but only recently did Kampschmidt *et al. (13)* show that a lipoprotein from egg yolk prevented cold-shock. Lecithin was also shown by Blackshaw *(3)* and by Lovelock *(14)* to have similar properties.

The effects of cold-shock on metabolism and the mode of action of protective substances have, in the past, received little attention. Earlier studies by Chang and Walton *(5)* showed that cold-shock greatly reduced the respiratory activity of ram semen. A brief notation by Hancock *(11)* also indicated that glycolysis, as well as the motility of bull semen, was reduced by cold-shock.

A recent approach to the problem was made by Mann and Lutwak-Mann *(15)*, who also showed that cold-shock of ram semen abolished both fructolysis and respiration. Adenosine triphosphate leaked from the cells and the ability of the cells to synthesize it was destroyed. Intracellular nitrogenous constituents, including cytochrome C and a lipoprotein, were lost.

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This paper presents evidence on the effect of cold-shock on the permeability of the sperm cell membrane, as revealed by the mineral content of the seminal plasma before and after cold-shock, the subsequent metabolic activity of bull semen, and the degree of protection against cold-shock provided by lecithin and egg yolk.

MATERIALS AND METHODS

Fresh bull semen of good initial motility was used in the tests. The semen samples contained approximately 200 ± 25 million spermatozoa per ml. after dilution. The range in final cell concentration was sufficiently standard, so as to permit calculation of the results in the conventional Z = activity/10^8 cells/hour. Each ejaculate was divided and subsamples subjected to each treatment for the particular experiments. The semen was diluted at room temperature (25° C.) as soon after collection as possible (usually within two hours), at a dilution rate of one volume of semen to four volumes of diluent.

The diluents used were *(a)* 0.9% NaCl, *(b)* a solution isotonic with *(a)*, prepared by mixing 0.13 *M* $Na_2HPO_4 \cdot 12\ H_2O$ and 0.17 *M* $NaH_2PO_4 \cdot H_2O$ to a pH of 7.0, *(c)* an isotonic medium of pH 7.0 (buffered NaCl) containing 0.032 *M* $NaH_2PO_4 \cdot H_2O$, 0.048 *M* $Na_2HPO_4 \cdot 12\ H_2O$, and 0.04 *M* NaCl; in some tests 0.022 *M* fructose was added *(25)*, and *(d)* 2.9% sodium citrate dihydrate. Motility estimates of the rate and the percentage of motile spermatozoa were made at the beginning and at the end of all experiments *(2)*.

The initial and final pH were determined, using a Beckman glass electrode pH meter. Aliquots of semen were taken at the beginning and end of the tests, for the estimation of fructose, reducing substances, and lactic acid. The methods used for these estimations, and also for the determination of oxygen uptake, have been described in an earlier paper *(4)*.

Cold-shock was produced by plunging samples of diluted, or in some cases undiluted, semen at 25° C. into ice water and holding them at 0° C. for ten minutes. After the shock treatment, the semen was rewarmed to 25° C. One-ml. aliquots of diluted semen, both shocked and unshocked, were transferred to Warburg flasks and incubated at 21 and 37° C. for eight and four hours, respectively, in the first tests; subsequently, only 37° C. and an incubation time of four hours were used.

To study the effect of protective substances on the occurrence of cold-shock, 0.5% lecithin and 20% egg yolk suspensions were used. Lecithin was prepared from a soybean extract, using essentially the method of Pangborn *(19)*.

To determine some of the possible effects of cold-shock on the permeability of the membrane surrounding the sperm cell, chemical analyses of certain constituents of seminal plasma were made before and after cold-shock of fresh semen. One-ml. aliquots of fresh semen were pipetted into two centrifuge tubes at 25° C. One tube was kept at 25° C. and the other was shocked at 0° C. for ten minutes. After a further 15 minutes at 25° C., both tubes were centrifuged and the supernatant plasmas removed for analysis. Sodium, potassium, and calcium were estimated with a Coleman Model 21 flame photometer, calibrated by

the methods outlined by Cragle, VanDemark, and Salisbury *(7)*. Inorganic and ether alcohol–extractable phosphorus in the plasma was estimated by the method for lipid phosphorus described by Hawk *et al. (12)*, involving oxidation of the extracted lipids and determination of the total phosphorus by the colorimetric method of Fiske and SubbaRow *(9)*. The electrometric method of Chanin *(6)* was used to determine chloride, and the osmotic pressure of the seminal plasma was measured by a Fiske osmometer, which employs a thermistor element to measure the depression of the freezing point.

RESULTS

The effects of cold-shock on the characteristics and chemical constituents of the seminal plasma are presented (Table 1). The data are presented so as to show the change in the cells. An increase in the potassium content of the plasma

TABLE 1

Effects of cold-shock of undiluted semen on the constituents of the seminal plasma

Constituent	No. obs.	(*a*) Control	(*b*) Cold shock	(*c*) Difference (*a*—*b*)
Osmotic pressure (*m. osm.*)	14[a]	329	334	−5
Sodium (*mg. %*)	32	209	199	10[b]
Potassium (*mg. %*)	30	243	260	−17[b]
Calcium (*mg. %*)	32	28	26	2[b]
Chloride (*mg. %*)	18	214	212	2
Lipid phosphorus (*mg. %*)	14	49	52	−3[b]

[a] Number of ejaculates.
[b] $P \leqq 0.05$.

of cold-shocked semen reflected a loss of this element from the cells. This fact is shown [Column (*c*), Table 1] by a minus sign before the figure. The statistical significance of changes observed were tested by the "t" test. The uptake by the shocked cells of sodium ($t=3.3$, $P=<0.01$) and of calcium ($t=7.5$, $P=<0.01$); and the loss of potassium ($t=4.3$, $P=<0.05$) and lipid phosphorus from the cells ($t=2.6$, $P=<0.05$), were the significant changes measured.

Results of the experiments to test the effects of cold-shock on the subsequent metabolism of semen diluted 1 : 4 with 0.9% NaCl and with the isotonic phosphate diluent are given (Table 2).

That table gives the mean hourly changes in fructose, lactic acid, and oxygen for both the control and the shocked semen. Analyses of variance showed marked differences in the rates of metabolism of shocked and control semen when incubated at 37° C. Variance ratios for fructose, lactic acid, and oxygen were 34.8, 15.9, and 154.7, respectively, giving, with one and six degrees of freedom, $P < 0.01$ in all cases. However, at 21° C., after eight hours of incubation, there were no significant metabolic differences between the two treatments.

The uptake of oxygen at 37° C. was greater in saline than in phosphate [$F_{(1, 6)} = 33.4$, $P < 0.01$] for the control semen, but after cold-shock no significant difference was noted.

TABLE 2

Aerobic metabolism of unwashed bull semen incubated at 21 and 37° C. after cold-shock in saline and phosphate diluents (means of 7 ejaculates)

Treatment	Diluent	Aerobic Z[a] Values of Semen			+Z LA / −Z Fruct.
		−Z O_2	−Z Fruct.	+Z Lactic Acid	(× 100)
		(μl.)	(μg.)	(μg.)	(%)
		A. 21° C.			
Control	NaCl	1.2	0.5	−0.5	—
Control	$HPO_4^=$	1.1	5.1	1.5	30
Shocked	NaCl	0.8	0.9	0.7	78
Shocked	$HPO_4^=$	0.7	1.5	3.1	200
		B. 37° C.			
Control	NaCl	8.6	19.7	14.5	74
Control	$HPO_4^=$	4.4	32.9	23.9	73
Shocked	NaCl	1.9	2.7	5.9	218
Shocked	$HPO_4^=$	1.7	2.4	7.9	329

[a] Z = Activity/10^8 cells/hr.

Although there were no significant metabolic differences at 21° C. between shocked and unshocked semen, the motility of the semen immediately after the shock was very low, and it fell even lower after incubation.

It may be noted here that cold-shock produced changes in the ratio of lactic acid accumulation to fructose utilization. This is usually less than one, but in these experiments the ratio exceeded 2.0 in the case of phosphate at 21° C., and both NaCl and phosphate at 37° C.

Further metabolic experiments were conducted at 37° C. to test the effects of lecithin and egg yolk on the occurrence of cold-shock. The lecithin (0.5%) was suspended in the buffered saline (C) described earlier and the egg yolk (20%) was mixed with the buffered saline or 2.9% sodium citrate. The lecithin test was further modified by the addition of 400 mg.% fructose to one-half the experimental lots. Oxygen uptake was measured after the standard cold-shock treatment only in the lecithin series, in which corrections were made for any oxygen change produced by the lecithin itself.

Data for the lecithin series are given (Table 3). The analysis of variance proved that lecithin protected the fructolytic capacity of the cells against cold-shock [$F_{(1, 12)} = 13.0$ and 12.3 for fructose utilization and lactic acid accumulation, respectively, $P = <0.01$]. However, no significant protection of the respiration function resulted. The addition of fructose reduced fructose utilization more in the absence than in the presence of lecithin, with both the control and cold-shocked semen. In the case of the cold-shocked semen, there was more fructose at the end than at the beginning of incubation.

This may be solely an effect of cold-shock on cell permeability, permitting leakage from the cells. The pH of the mixtures was effectively buffered, the means being 6.7 ± 0.1 at the end of the incubation for all samples.

TABLE 3

Aerobic metabolism of bull semen at 37° C. after cold-shock in the presence or absence of 0.5% lecithin and of fructose (means of 6 ejaculates)

Buffered saline diluent		Aerobic Z[a] Values of Semen			+Z LA
Lecithin addition	Fructose addition	−Z O_2	−Z Fruct.	+Z Lactic Acid	−Z Fruct. (× 100)
(%)	*(mg. %)*	*(μl.)*	*(μg.)*	*(μg.)*	*(%)*
		A. Control			
0	0	4.6	38.0	24.7	65
0.5	0	4.7	30.5	25.1	82
0	400	3.9	4.4	22.4	509
0.5	400	3.5	27.4	31.6	115
		B. Shocked			
0	0	1.9	−0.1	6.9	—
0.5	0	3.5	21.4	18.9	88
0	400	1.6	−21.4	6.4	—
0.5	400	1.9	18.8	19.7	105

[a] Z = Activity/10^8 cells/hr.

Results showing the protective action of egg yolk against cold-shock are summarized (Table 4). Egg yolk clearly had a highly beneficial effect on metabolism after cold-shock, and it also had a general stimulating action on the metabolism of the control semen (variance ratios for lactic acid and reducing substances were 109.9 and 31.6, respectively, with one and 21 degrees of freedom, giving $P < 0.01$ in both cases). In the presence of egg yolk, motility also was maintained well after cold-shock, at a rate close to that of the control semen.

DISCUSSION

It has been shown above that the aerobic glycolysis and respiration were indeed greatly reduced by cold-shock, but that the effect was considerably in-

TABLE 4

Aerobic glycolysis of bull semen at 37° C. after cold-shock in the presence or absence of egg yolk (means of 6 ejaculates)

Diluent	Egg yolk	Z[a] Values of Semen		+Z LA
		−Z Red. Subst.	+Z Lactic Acid	−Z Red. Subst. (× 100)
	(%)	*(μg.)*	*(μg.)*	*(%)*
		A. Control		
Buffered saline	0	68.0	55.1	81
Sodium citrate	0	79.8	48.8	61
Buffered saline	20	118.6	81.9	69
Sodium citrate	20	138.6	89.0	64
		B. Shocked		
Buffered saline	0	12.0	18.8	157
Sodium citrate	0	24.6	15.9	65
Buffered saline	20	104.0	67.1	64
Sodium citrate	20	98.2	81.1	82

[a] Z = Activity/10^8 cells/hr.

fluenced by the subsequent incubation temperature. At 37° C., the inability of shocked cells to maintain their metabolic functions was clearly seen, but at 21° C. there were no significant metabolic differences between the shocked and the control semen, suggesting that the loss of vital cell constituents is increased by the higher temperature of subsequent incubation.

A moderately constant feature following cold-shock was an increase in the ratio of lactic acid accumulation to sugar loss. Usually, this is less than one, but after shock it may exceed two. This was due rather to a greater relative effect of depressing the measured sugar utilization than to an increase in the accumulation of lactic acid, which in turn may reflect the production of lactic acid from another substrate and/or formation of fructose or leakage of fructose from the shocked cell.

Substances which protect semen from cold-shock, such as egg yolk and lecithin, repeatedly have been shown to prevent the decline in motility, but their corresponding effects on metabolism have been little studied. The present study has shown that both these substances reduced the damage to glycolysis produced by shock, although egg yolk was more efficient. Egg yolk also stimulates the glycolytic activity of unshocked semen and after shock maintains it at a level higher than control semen without yolk. The activation of the succinic and malic dehydrogenases of bull spermatozoa by egg yolk has been reported by Smith, Mayer, and Merilan *(23)*, who suggest that the factor responsible is related to the acetone-soluble fraction of the yolk, particularly carotene and cholesterol, because these two compounds in cell preparations devoid of yolk stimulated the succinate dehydrogenase system. However, Tosic and Walton *(24)* found that the respiration of bull semen was enhanced by the fat-free protein residue of the nondialyzable part of egg yolk as well as the fat.

The mechanism of the protective action of substances like egg yolk and lecithin is not known, but it seems reasonable to suggest that at least a part is a surface property which maintains the integrity and function of the cell membrane.

The concentrations of some cations in semen and seminal plasma have been examined by Salisbury and Cragle *(21)*, who found that potassium levels were higher in the cells and that sodium and calcium levels were higher in the seminal plasma. The changes in ionic concentrations in the seminal plasma following shock suggest a breakdown in the selective permeability of the cell membrane, followed by a passive transfer in the directions indicated by the concentration gradients. The maintenance of a concentration in the cell against these gradients is dependent on metabolic processes which are disrupted by cold-shock.

These observations extend those already reported on the effects of cold-shock on ram semen by Mann and Lutwak-Mann *(15)*, and in bull semen, by Mayer *(16)*. The first *(15)* of these two reports emphasized the rapid loss from cold-shocked cells of adenosine triphosphate and the inability of the shocked cells to synthesize this important energy-mediator. A slower process was the leakage of the respiratory enzyme, cytochrome C, and of nitrogenous substances and a lipoprotein from the cells. The second *(16)* emphasized that the degree and duration of cold-shock influenced the amount of metabolic damage.

SUMMARY

Rapid cooling of diluted bull semen from 25 to near 0° C. (cold-shock) produced a marked loss of motility, respiratory activity, and glycolysis. The motility and metabolic damage produced by rapid cooling could be partially prevented by lecithin and almost completely so by egg yolk. The egg yolk also stimulated the glycolysis of unshocked semen. Cold-shock of whole semen caused a loss of potassium and ether alcohol–extractable phosphorus from the cells into the plasma, and an uptake of sodium and calcium by the cells.

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EFFECT OF DIETARY AND MANAGEMENTAL FACTORS ON REFLEX CLOSURE OF THE ESOPHAGEAL GROOVE IN THE DAIRY CALF[1]

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Effect of dietary and managemental factors on the point of deposition in the stomach of liquids and capsules, fed by the nipple- and bucket-method to four fistulated calves, were studied. Capsules $\frac{5}{8} \times 1\frac{5}{8}$ inches passed readily through the esophageal groove to the omasum when it was closed by concomitant feeding of liquids. Nearly all capsules, regardless of size, were deposited in the reticulum when no liquids were consumed. Consumption of liquids closed the esophageal groove in all calves the first six weeks after birth. Nipple-feeding caused closure of the groove for at least 13 weeks, whereas bucket-feeding was less effective after six weeks. *Editor.*

The function of the esophageal groove (a muscular structure extending downward from the cardia to the omasum on the median wall in the reticulum) has been studied by many investigators. An extensive review of relevant literature and of studies on deglutition in sheep has been presented by Watson *(5)*. Over a century ago, the esophageal groove was thought to be concerned with bolus-formation and regurgitation. Flourens *(1)* first disproved this hypothesis; later Flourens' conclusions were substantiated by Wester *(6)*. Schalk and Amadon *(3)*, using young rumen-fistulated calves, found when a nipple device was employed that most of the milk was directed through the groove into the omasum and abomasum; however, when the calves drank from an open bucket, a large portion of the milk gushed directly into the rumino-reticular cavity. Wise *et al. (9)*, working with fistulated calves from 17 to 56 days of age, also found more milk entering the rumino-reticular cavity when the calves were fed from an open bucket than when fed from a nipple-pail. Watson *(5)* concluded that passage to the abomasum of liquids sucked by lambs is due not to the act of sucking itself but rather to the activation of the pattern of behavior which surrounds the act of sucking.

Further study seemed desirable, since the effects of the open-bucket and nipple-pail methods of feeding, and of various types of liquids on closure of the esophageal groove, are not entirely clear. Moreover, it frequently is desirable to by-pass the rumino-reticular cavity with therapeutic agents. Ross *(2)* demonstrated that a 1.0% solution of $CuSO_4$ effected closure of the esophageal groove of ruminants. Likewise, Watson *(5)* observed that a solution of $CuSO_4$ (and also solutions of cupric acetate and cupric chloride) cause closure of the groove. However, other simple, efficient means of routing materials directly

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into the omaso-abomasal cavity seemed to warrant examination. The objective of the present study was to clarify further the effects of various dietary and managemental factors on the initial point of deposition in the stomach, of various liquids and capsules fed to the calf.

EXPERIMENTAL PROCEDURE

Four male calves provided with rumen fistulae were used in this study. The rumen fistula operation was initiated at nine days of age for one calf and at 18 days for the other three. Each fistula was prepared in two stages. In the initial step, an area of the rumen about two inches in diameter was exposed, and the edge of this area was sutured to the skin in the paralumbar fossa area. Four days later the exposed rumen tissue was removed. After the final incision, a rubber "sleeve" was fitted into the fistula. The sleeve was prepared by gluing each end of a cylindrical piece of rubber dam to a 5-inch V-belt as shown (Figure 1). The sleeve when inserted (Figure 2) protected

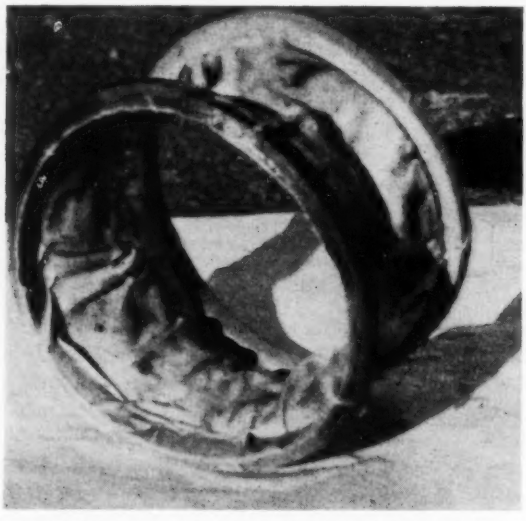

FIG. 1. Rubber sleeve used to protect the edge of the fistula.

the edge of the fistula and stretched the aperture to approximately 3½ inches in diameter, a size maintained throughout the study. The fistula opening was closed by placing a plastic bowl-cover over the exterior end of the sleeve. This was an adequate means of closure as long as the calf was maintained on liquid diets. When hay and dry concentrates were fed, a wooden plug was used, to prevent excessive loss of ingesta through the fistula.

The calves were confined to individual pens in a calf barn maintained at a minimum temperature of 60° F. Except when experimental diets were

FIG. 2. Rubber sleeve placed in fistula of Calf 3821.

fed, the calves received whole milk (about 3.4% fat) at the rate of ten to 12 pounds per 100 pounds body weight. This milk was fed by nipple-pail in three equal feedings per day (6 A.M., noon, 6 P.M.). Each calf received weekly 140,000 I.U. of supplemental vitamin A added to the milk and 9,000 I.U. of vitamin D by capsule. A trace-mineral mixture was given by capsule, but administration was not concurrent with test-meal feeding.

The effects of whole milk (approximately 3.4% fat), reconstituted skim-milk, reconstituted whey product,[3] and water on the closure of the groove were evaluated. Four sizes of gelatin capsules (ranging from ¼- to ½-inch in diameter and from ¾-inch to 1⅛ inches in length) were administered with and without the various liquids. Both the nipple-pail and the open-bucket systems of feeding were employed. The method of feeding and order of placing each calf on the various liquids were determined by using a randomized Latin-square design. The randomization and order of administration of capsules were determined from tables of random numbers *(4)*.

The calves were placed on experiment for four consecutive days, during which time each of the liquids was fed for one day. Capsules were administered on each of these days. The liquid assigned for each specific day was fed at the morning and evening feedings, at which times observations were made. One experimental feeding each day was by nipple-pail, the other by open bucket. Whole milk was fed by nipple-pail at the noon feeding, to provide a more adequate diet. At the end of the four-day experimental period, the calves were fed whole milk by nipple-pail for three days, to serve as an adjustment period; then the experimental feeding regime was repeated.

During each series of capsule administration with liquid, eight capsules, two of each of the four sizes, were introduced into the mouth by hand during the time the liquid was being consumed. Visual or manual examination of the esophageal groove was made during each administration, to determine the initial point of deposition (rumino-reticular cavity or omaso-abomasal area). Approximately midway between the noon and evening feeding, the same number

[3] From dried whey product, supplied by Western Condensing Company, Appleton, Wisconsin.

of capsules as used in milk-feeding trials were administered by hand, with no liquid, and similar examinations were made to ascertain the point of deposition.

At 45 days of age, the open bucket was substituted for the nipple-pail in the nonexperimental feedings of two calves (No. 3820 and No. 3821); nipple-pail feeding was continued for the other two (No. 3814 and No. 3817). Hay, dry concentrates, and water were fed free choice to Calf 3817 subsequent to 64 days and to Calf 3820 after 81 days. Calves 3814 and 3821 received no hay or grain until they were 125 days of age. Examinations were made periodically, employing the four liquids and both nipple-pail and open-bucket methods, and the strength of closure of the groove was noted. The experiment was terminated when the calves reached five months of age.

RESULTS AND DISCUSSION

Effects of various liquids upon the reflex closure of the esophageal groove and the initial point of deposition of capsules are indicated (Table 1). The assumption was made that a capsule had passed through the groove if it did not appear in the rumino-reticular cavity within approximately one minute after administration. In most instances, passage of the capsule through the groove could be detected by palpation of the area of the groove via the ruminal fistula.

A high degree of uniformity was noted among calves treated similarly. During the oral administration of 512 capsules, while calves were consuming a liquid concurrently, only two capsules were deposited in the rumino-reticular cavity. These two capsules were deposited in the reticulum when Calf 3814 paused while drinking water from an open bucket. The point during a meal at which the capsule was given had little effect upon response, as long as the calf was drinking during capsule administration. These data indicate that in the young calf, the type of liquid, method of feeding, and size of capsule (within the range and under the conditions employed) have no effect upon the initial point of deposition, if a calf is consuming a liquid at the time a capsule is administered. Furthermore, these results demonstrate the feasibility of direct administration of capsules containing vitamins and various therapeutic agents into the omaso-abomasal cavity. In the case of vitamins, this procedure is highly desirable, since some vitamins (such as carotene and vitamins A and E) may be partially destroyed (by oxidation or otherwise) in the rumino-reticular area of the young calf.

Of the 256 capsules administered to the calves without a liquid, 251 appeared in the reticulum. Of the remaining five capsules, four were small (No. 1, Table 1) and might have been retained in the mouth of the calf until dissolved. No contraction of the groove was observed during any of these administrations in the absence of a liquid. This is in accordance with the findings of Ross *(2)*, who administered glass beads up to ⅛-inch in diameter with various solutions. He also found the passage of these particles through the groove to be dependent upon the same factors that determined the passage of the fluids alone. Wise *(7)* reported that drinking tap-water from an open bucket stimulated closure of

TABLE 1

Initial place of deposition of capsules administered with and without various liquids to calves ranging from 20 to 36 days of age

Liquid	Size of capsule							
	1[a] No. deposited in:		2[b] No. deposited in:		3[c] No. deposited in:		4[d] No. deposited in:	
	Reticulum	Omasum	Reticulum	Omasum	Reticulum	Omasum	Reticulum	Omasum
Water	0	32	0	32	1	31	1	31
Whey[e]	0	32	0	32	0	32	0	32
Skimmilk[f]	0	32	0	32	0	32	0	32
Whole milk	0	32	0	32	0	32	0	32
Total liquid	0	128	0	128	1	127	1	127
No liquid	60	4	64	0	63	1	64	0

[a] Capsule size, ¼-in. diameter and ¾-in. length.
[b] Capsule size, 5/16-in. diameter and ⅞-in. length.
[c] Capsule size, ⅜-in. diameter and 1-in. length.
[d] Capsule size, ½-in. diameter and 1⅛-in. length.
[e] Reconstituted whey product (one part whey product: nine parts water).
[f] Reconstituted skimmilk (one part dried skimmilk: nine parts water).

the esophageal groove of calves until they were three or four weeks of age, after which the response began to subside. In the present study, the groove was stimulated (under rather similar conditions) to about eight weeks of age. The reason for the difference is not clear, but may have been associated with variations in behavior patterns of the calves, or differences in management. The present observations also are in contrast to the findings of Schalk and Amadon *(3)*, who reported that much of the milk consumed from an open bucket passed directly into the rumino-reticular cavity.

In most instances, up to eight weeks of age the closure of the groove was rapid and complete during the consumption of each of the aforementioned liquids. It was further observed that the pillars of the groove are relatively strong. Large capsules (⅝-inch in diameter and 1⅝ inches in length) were administered occasionally during this period, and by manual palpation of the esophageal groove the capsules were detected as they passed through the groove to the omaso-abomasal cavity. After drinking ceased, the length of time required for the pillars of the groove to relax varied among the several animals, and even in the same animal, from approximately ten seconds to more than one minute.

The age at which esophageal groove function became inefficient under the various conditions is shown (Table 2). During the period of observation, water was less effective than the other liquids. Also, the open bucket was not as effective as the nipple-pail in stimulating closure of the groove. The presence of feed (hay and grain concentrates) in the rumino-reticular cavity of Calves 3817 and 3820 had no appreciable effect upon the closure of the groove. Calves 3820 and 3821 were fed the nonexperimental diet by open bucket after 45 days of age, a change which seemed to have no major effect on efficiency

TABLE 2

Age at which esophageal groove closure became inefficient[a]

Liquid	Method of feeding[b]	Calf No. 3814	3817	3820	3821	Mean
		(age, weeks)				
Water	O.B.[c]	11	9	7	6	8
	N.P.[d]	14	13	16	16	15
Whey	O.B.	11	9	14	14	12
	N.P.	>20	>20	>20	>20	>20
Skimmilk	O.B.	11	9	14	14	12
	N.P.	>20	>20	>20	>20	>20
Whole milk	O.B.	11	10	16	16	13
	N.P.	>20	>20	>20	>20	>20

[a]Estimated 25% or more spillage to the rumino-reticular cavity.
[b]Nonexperimental feedings for all calves were by nipple-pail to 45 days of age; after that time, nonexperimental feedings of Calves 3820 and 3821 were from open bucket. Hay, dry concentrates, and water were fed free choice to Calf 3817 subsequent to 64 days and to Calf 3820 after 81 days.
[c]O.B. — Open bucket.
[d]N.P. — Nipple pail.

of the groove. There was, however, a suggestion that feeding from the open bucket improved groove function when whey, skimmilk, and whole milk were consumed from the open pail. This is not in accord with the results of Wise *et al. (8, 9)*, who found a more complete closure of the groove in calves 17 to 176 days of age fed by nipple-pail than in those fed by open bucket. This difference may have been due to variations in experimental techniques between the two studies. Further study may be necessary to clarify the factors involved.

Subsequent to 125 days, all calves in the present study were fed hay and a concentrate mixture in addition to milk. Three weeks later, when all calves showed marked rumen development, closure of the esophageal groove of each calf occurred when stimulated by nipple-pail feeding of whole milk, skimmilk, or whey.

Further study is needed to define more clearly the effects of various managemental practices. It has been concluded by Watson *(5)* that passage of liquids to the abomasum of lambs is not influenced by temperature or composition of the liquids observed, by posture assumed by the animal while nursing, nor by the act of sucking but rather by activation of the pattern of behavior which surrounds the act of nursing. In the present study, however, water was definitely less effective than the other liquids. It was observed in the study reported herein that the presence of attendants, who fed the calves, in the vicinity of the pens at feeding time sometimes resulted in closure of the esophageal groove. These observations lend further support to the contention that behavior patterns and conditioned responses are important in the functioning of the esophageal groove.

SUMMARY

Rumen fistulae were prepared in four young calves. The calves were fed the experimental diets either by open bucket or nipple-pail; however, only the nipple-pail was used in feeding the nonexperimental diet for 45 days.

At this time, two of the calves were changed to the open-bucket method of feeding of the nonexperimental diet, whereas the other two were continued on nipple-pail feeding. One of each pair was also fed hay, dry concentrates, and water free choice after 64 and 81 days of age, respectively. The remaining two calves were placed on a hay-and-grain ration after 125 days of liquid feeding.

Capsules of various sizes ranging up to 5/8-inch in diameter and 1 5/8 inches in length passed readily through the groove to the omasum, when the groove was closed by concomitant feeding of the various liquids. Nearly all capsules, regardless of size, were deposited in the reticulum when no liquid was consumed concurrently by the calf.

All of the liquids studied (whole milk, reconstituted skimmilk, reconstituted whey product, and water) effected complete closure of the esophageal groove in all calves during the first six weeks after birth. Moreover, to six weeks of age the two methods of experimental feeding, nipple-pail and open-bucket, were equally efficient. Nipple-pail feeding effected closure of the groove in all calves up to 13 weeks of age (in some cases longer), whereas open-bucket feeding was less effective subsequent to six weeks.

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LACTOSE IN ANIMAL AND HUMAN FEEDING: A REVIEW

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Lactose is found only in milk. It is a reducing disaccharide which on hydrolysis yields equal quantities of glucose and galactose. Since lactose has been shown to exhibit mutarotation, it exists in both the alpha and beta form. It is formed in the mammary gland from glucose or glycogen and is not notably changed by modifications of the maternal diet, or by the level of blood sugar. The percentage of lactose in milk varies significantly for different species. Human milk has a higher lactose content than the milk of dairy animals. When babies are reared on cow's milk, it is customary to add some carbohydrate such as lactose, sucrose, glucose, maltose, or dextrin. Compared to other sugars, lactose is less soluble and less sweet. This lack of sweetness makes it ideal for feeding infants and invalids, because it does not retard the appetite. Lactose is less readily hydrolyzed in the intestine than other disaccharides, and some of it is absorbed and reaches the blood stream intact. Because of its slow digestion and absorption, lactose reaches a much lower portion of the large intestine than other sugars and is, therefore, more effective in promoting the growth of acidophilic organisms.

This review will summarize some of the results obtained with lactose by research workers in animal and human nutrition, since it was believed this would be of interest to workers in these fields.

GENERAL

Experiments by Schatti *(90)* and by Ariyama and Takahashi *(2, 3)* indicated that lactose is not absorbed as well as other carbohydrates, and causes only a slight increase in the blood sugar level. In tests to determine the effectiveness of carbohydrates in promoting the growth of rats, these workers showed that, in spite of its low absorption and assimilability, lactose was very effective in promoting growth of animals, especially during the early part of the feeding period.

Greisheimer and Johnson *(42)*, Fisher *(26)*, Whittier *et al. (99)*, Mitchell *et al (66)*, Ershoff and Deuel *(23)*, Handler *(44)*, and Scheunert and Sommer *(91)* were able to show that rats do not tolerate rations in which lactose is the sole source of carbohydrate. Such animals develop a diarrhea and alopecia. Animals fed lactose as the sole source of carbohydrate may or may not develop a tolerance after receiving such a diet for some time.

Koehler and Allen *(55)* reported that rats were able to tolerate a ration containing 35% lactose. These rats gained better than rats on rations containing other carbohydrates, during the early part of the experimental period, but had lost their advantage by the tenth day of the experiment. These workers

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reported that approximately 50% of the lactose ingested was excreted. Riggs and Beaty *(81)* were able to show that growth and food utilization were normal when diets fed to rats contained no more than 15% lactose. When the diet contained 20 to 30% lactose, transitory symptoms of lactose feeding (diarrhea and/or "pot bellies") appeared. However, growth and food utilization were normal. When 30% or more of lactose was fed, definite symptoms of lactose feeding were observed.

Ershoff *(22)* studied the nutritive value of lactose by using the single-food method. Rats fed a diet composed solely of lactose survived for an average of five to seven days; whereas, rats receiving other carbohydrates (dextrose or sucrose) survived for an average of 34 days. Scott and Verney *(92)* gave rats a choice of carbohydrate and reported that they avoided lactose when other carbohydrates were available.

Experiments by Le Gallic *(59)* indicated that the maximum quantity of lactose which could be metabolized by the rat was in constant relation with the other organic factors in the diet, regardless of what the other foods might be. This was also shown by Lecoq *(58)*, who demonstrated that rats tolerated larger amounts of lactose when the diet contained greater quantities of the B-vitamins.

Coryell and Christman *(16)* showed that the fasting rat was able to hydrolyze 73% of a dose of lactose given by stomach tube, and that some glycogen was formed from the lactose hydrolyzed.

LACTOSE, AND CATARACT FORMATION IN RATS

The formation of cataracts in the eyes of rats fed diets high in lactose has been reported by a number of workers. Mitchell and Dodge *(69)* reported a high incidence of cataract in the eyes of rats fed rations containing 70% lactose. Rations containing smaller quantities of lactose resulted in slower development and a less pronounced type of lens change. None was reported when the diet contained 30% or less of lactose. This was substantiated by Yudkin and Arnold *(100)*, who reported cataract formation on rations containing 70% lactose or 35% galactose.

Morgan and Cook *(72)* also reported the formation of cataracts in the eyes of rats fed diets high in lactose, but reported that the formation of the cataracts took three times as long when the diets contained a considerable quantity of vitamin G. Mitchell *et al. (70)* produced cataracts on rations high in lactose or galactose. These workers concluded that galactose was the sugar responsible for the high incidence of cataract, when such rations were fed.

Work by Borley and Tainter *(13)*, on the effect of dinitrophenol on the cataract development in rats given rations high in lactose, indicated that dinitrophenol had no effect on cataract formation. Yudkin and Geer *(101)* reported that the development of the cataract produced by high lactose or galactose feeding could be retarded by feeding a diet high in protein. They also reported that a high intake of cystine also tended to retard the pathological changes, but that massive doses of riboflavin or liberal amounts of the vitamin B–com-

plex, in the form of brewers yeast, exerted no protective action against the development of galactose cataract.

An excellent review of the literature on lactose and galactose cataract was published by Hormann *(48)*. This author reported that lactose and galactose apparently hindered the fermentative carbohydrate metabolism, and that the injection of cocarboxylase prevented the toxic action of the sugars and largely prevented the formation of cataract by galactose. It was postulated that the origin of the cataract could be due to a disturbance of the conversion of pyroracemic acid into lactic acid in the lens.

SWINE FEEDING

In contrast to rats, pigs appear to have a much higher tolerance for lactose. Becker *et al. (10)* studied the nutritive value of dextrose, lactose, sucrose, dextrin, and cornstarch for the newborn pig, by using a synthetic milk diet. From seven to 35 days of age dextrose, lactose, dextrin, and cornstarch produced equal body-weight gains and survival. The feed efficiency was found to be similar except for lactose, which was superior. Forty per cent of the pigs fed sucrose died from severe diarrhea. Further work by Becker and Terrill *(9)* indicated that the older pig was not able to tolerate lactose in large amounts in comparison with the newborn pig. Pigs nine weeks of age were able to tolerate a ration containing 50% lactose, though a moderate diarrhea was noted. Sixteen-week-old pigs were able to tolerate 25% lactose without harmful effects.

Catron *(15)* studied 11 carbohydrates as the sole source of carbohydrate for baby pigs one to five weeks of age. Lactose gave outstanding results both in growth and feed efficiency. Partially dextrinized starch (D.E., dextrose equivalent 10), which gives best performance with the rat, was poorest for the baby pig.

Golding and Morris *(40)* studied the use of two by-products of lactose manufacture, Lactose Paste and Lactose Feed. Pigs fed 10% of either of these products showed improved growth and feed efficiency. The general appearance of the pigs was improved by the whey residues, as were also the pork and bacon produced from pigs fed these products.

DAIRY-CALF FEEDING

Rojas *et al. (85)* studied the effect of feeding whole milk and modified milk diets to dairy calves. Calves fed whole milk or skimmilk for periods up to 42 days utilized the dietary lactose very effectively. When the lactose content of the milk was doubled, there resulted an increase in urinary galactose equivalent to 8% of the lactose ingested. This galacturia was accompanied by diarrhea and unthriftiness of the animals.

Flipse *et al. (27, 28)* studied the use of synthetic milk for dairy calves by using synthetic milks which varied only in the source of carbohydrate. For a 31-day experimental period the average gain for the glucose-fed animal (60% glucose) was 9.33 lb. When 30% of the glucose was substituted with corn syrup, the

gain was 8.66 lb. When 10% of the glucose was replaced with lactose, the gain was 8.66 lb. Feed efficiency was better in the groups fed lactose. In a second study by these workers, calves receiving a synthetic milk containing 5% glucose, 45% starch, and 10% lactose gained 24.67 lb. in 31 days. Calves on synthetic milk containing 15% glucose and 45% starch gained only 14.0 lb. for the same period. Feed efficiency was much better in the group receiving lactose.

POULTRY FEEDING

Poultry are apparently unable to utilize large amounts of lactose. Manson *et al. (64)*, in an experiment with chicks using rations containing 61% lactose, found that lactose was utilized to a lesser extent than was dextrin, cerelose, or sucrose. It was noted, however, that lactose-fed chicks gave greater responses to reticulogen, fish solubles, and high levels of vitamin B_{12}. Rutter *et al. (86)* reported that the inclusion of lactose in chick rations at levels up to 20% did not impair the growth rate or obviously affect their physiological well-being. Above 20%, lactose feeding caused growth impairment, diarrhea, and a "crooked-toe" deformity. These workers reported that galactose was well-tolerated at levels up to 10%. At higher galactose levels, a characteristic nervous syndrome was observed which was characterized by epileptiform convulsions.

Mature hens have less tolerance for lactose than young birds. Lenkeit and Becker *(60)* reported that approximately 50% of the ingested lactose was excreted unchanged and that it was not practical to feed lactose to laying hens. Couch *et al. (17)* reported that hens fed synthetic diets containing lactose as the sole source of carbohydrate ceased laying in from one to two weeks after being placed on the diet. High levels of lactose apparently had no effect on hatchability. Hamilton and Card *(43)* reported that the hen utilized 8 g. of lactose per day fairly completely, and that chickens would not voluntarily consume more than 8 g. of lactose daily when pure lactose was added to the feed.

Scott *(93)* presented evidence that 3.25% lactose had a deleterious effect upon growth and increased the incidence of enlarged hocks in turkey poults. He postulated that this effect might be due to an effect upon the microbiological flora of the poult.

LACTOSE, AND INTESTINAL ACIDITY

Since lactose is difficultly hydrolyzed or absorbed, and tends to remain in the intestinal tract for quite some time, it is understandable that it might tend to have an effect on the pH and on the intestinal flora of the digestive tract.

Hull and Rettger *(49)* found that 2 or 3 g. of lactose daily brought about a complete transformation of the intestinal flora of the rat within two or three days. The explanation of the favorable influence of lactose on the aciduric bacteria of the intestine must be found in the fact that when lactose is present, even in minute quantities, optimum cultural and environmental conditions are created for these particular organisms, without a corresponding favorable change

for the mass of other bacteria ordinarily present. These workers also reported that a high lactose diet markedly influenced the intestinal flora of man.

Mitchell *(67, 68)* was able to show with rats that diets with lactose as the sole source of carbohydrate promoted the best growth of acidophilus but the poorest growth of the animal. These experiments indicated that high percentage acidophilus persisted as long as the diarrhea, and that the flora, after prolonged lactose feeding, reverted to the putrefactive type. This work indicated that lactose, at the 30% level, permitted normal growth and at the same time promoted an aciduric type of intestinal flora. These same results were reported by Cruickshank *(19)*, to the effect that lactose changed the intestinal flora of rats to an aciduric type. This work showed that sucrose had a much less marked effect in this respect; whereas, maltose, glucose, galactose, and levulose produced no change in the intestinal flora.

Kessel *(52)* presented evidence that lactose produced a lowering of the pH of the colon contents, with an increase in the gram positive organisms, including *Bacillus acidophilus*. He also reported that in some instances lactose-feeding caused a reduction in the number of trichomonads and amoebae. Nath *et al. (74)* reported that for rats on a lactose diet, the total aerobic and anaerobic plate counts, as well as the numbers of coliforms and lactics, was much higher than the corresponding counts or numbers of the same organisms in the cecal contents of animals on diets containing either dextrin or sucrose.

Beach *(7)* reported that the pH of the cecal contents of chickens was changed from the normal range of from 6.0 to 7.4 to a range of from 4.4 to 5.6 by feeding sufficient quantities of whole sweet milk, milk cultures of *B. acidophilus*, milk cultures of *B. acidophilus* plus lactose, lactose alone, or dry skimmilk. An abnormal degree of acidity in the ceca was maintained constant by the individual administration of 1 or 2 g. of lactose twice daily at intervals of about eight hours. It was also reported by Beach and Davis *(8)* that chicks were afforded a considerable degree of protection against coccidial infection when a sufficient amount of lactose or dry skimmilk was added to the diet. It was assumed that the changing of the cecal contents to a more acid medium formed an environment which was unfavorable for the coccidia. Becker and Morehouse *(11)*, on the other hand, reported that lactose did not exert any restraining influence upon the numerical increase of the coccidian population in the alimentary tract of the mammal.

Johansson *et al. (51)* reported that the type of dietary carbohydrate influenced the microflora of fecal droppings from laying pullets ingesting a purified biotin-deficient ration. Lactose-containing diets encouraged a fecal coliform flora, but lactic acid bacteria proliferate extensively in the intestines of hens on such a diet. The most marked effect noted in the case of sucrose was a depressing action on fecal coliforms.

An excellent review of the literature concerned with the effects of lactose on gastrointestinal motility was presented by Fischer and Sutton *(25)*.

LACTOSE, AND VITAMIN SYNTHESIS

Since lactose causes extensive changes in the bacterial flora of the intestinal tract, and since considerable quantities of vitamins are synthesized by the bacteria of the intestinal tract, it is logical to think that the feeding of lactose might bring about changes in the needs/or excretion of some of the vitamins. A considerable amount of research has been done along this line.

Morgan *et al. (73)* reported that when lactose in sufficient amount is incorporated in the basal diet of rats, riboflavin and vitamin B_6 need not be given, since they are apparently elaborated in the intestines of the animals. The filtrate factor must be given, however, in order to obtain normal growth. In no case did dermatitis develop in lactose-fed rats, even when the proportion of lactose was lowered to 30%, but a large proportion of rats developed cataracts. The severity of the cataract occurrence was mitigated when all the vitamin B factors were supplied, and this prophylactic effect seemed to inhere in the filtrate factor, particularly.

Mannering *et al. (63)* were able to show that rats fed riboflavin-deficient diets, high in dextrin or lactose, were able to survive; this indicated a considerable intestinal synthesis of riboflavin. The total fecal output of riboflavin of rats receiving such riboflavin-low diets was determined and in general, those carbohydrates which decreased the amount of dietary riboflavin needed for growth were those responsible for the greatest quantities of riboflavin in the feces. The fecal excretion of riboflavin was greatest when lactose was fed, even though the growth-promoting effect of lactose was not great. Tepley *et al. (96)* studied the effect of carbohydrate on the intestinal synthesis of both niacin and folic acid in the rat. The addition of lactose or milk powder to the basal ration increased the synthesis of both niacin and folic acid. When phthalylsulphathiazole was fed, this increase did not occur.

Couch *et al. (18)* presented evidence that dextrin favored the intestinal synthesis of biotin in the mature fowl and that sucrose did not promote such synthesis. Results obtained with lactose and dried whey indicated that lactose did not stimulate the synthesis of biotin in the intestinal tract of the laying hen.

The effect of sucrose, lactose, galactose, and fructose on the fecal excretion of biotin, riboflavin, and folic acid was studied in the mature cotton-rat by Bacigalupo *et al. (5)*, who reported that lactose promoted the intestinal synthesis of the three B-vitamins to an appreciable degree in all instances. Galactose promoted such synthesis, but to a much less degree than did lactose. Sucrose and fructose produced some apparent intestinal synthesis of biotin and folic acid.

De and Ray *(20)* reported on the effects of lactose, starch, and sucrose, as concerned with the biosynthesis of riboflavin in rats fed diets free of riboflavin. From the study of the urinary and fecal elimination of riboflavin in adult and young rats, with and without sulphaguanidine supplement, it was found that lactose was more effective than starch or sucrose in promoting the biosynthesis of riboflavin in the intestine of such rats. This intestinal synthesis was found to be greater in young than in adult animals.

FAT AND LACTOSE UTILIZATION

The relation of fat to the utilization of lactose has been of interest and has been investigated by a number of workers. Couch *et al. (17)* reported that laying hens, fed a purified diet with lactose as the sole source of carbohydrate, ceased laying in from one to two weeks after being placed on the diets. Thirty per cent fat in the diet corrected this deleterious effect of lactose on egg production. Schantz *et al. (89)* reported that when rats were placed on a mineralized skimmilk diet, sugar was readily detected in the urine after a few days of feeding. This sugar was identified as galactose and accounted for all the reducing material in the urine. It was also reported that fats such as butter fat, lard, corn oil, coconut oil, linseed oil, and palmitic and oleic acids, when added to the mineralized skimmilk at levels of 3 to 4%, prevented this loss in the urine.

Work by Ershoff and Deuel *(24)* indicated that the survival time of rats fed diets containing 70% lactose and 30% fat was greater than when the diet contained only lactose. This was not found to be true when sucrose was the carbohydrate. This work was confirmed by Venkatasubramanian and De *(97)*, who found a survival time of 61 days for rats fed a diet of groundnut (peanut) oil with 5% galactose added, as compared to 28 days for groundnut oil alone and eight days for galactose alone.

LACTOSE, AND TISSUE PHOSPHOLIPIDS

The relation of the diet to the composition of the tissue phospholipids was studied by Artom and Fishman *(4)*. The lipid composition of the livers of rats maintained on low-protein diets, in which the carbohydrate component consisted of equal parts of dextrin and sucrose, was compared with that of the livers of rats fed similar diets but in which lactose was substituted for sucrose. Smaller amounts of neutral fat were found in the livers from the lactose-fed animals. The effectiveness of choline in reducing the fat infiltration and in raising the lecithin level in the liver appeared greater in animals fed lactose-containing diets.

Sadhu *(87)* studied the effect of dextrose (1 gm/day) and lactose (1 gm/day) feeding in the pigeon. He noted an increase of galactocerebrosides and a decrease of sphingomyelin in lactose-fed pigeons; whereas, there was an increase of glucocerebrosides in dextrose-fed pigeons. Dehydrogenase activity (succinate and lactate) was decreased in the liver and increased in the intestine of lactose-fed pigeons.

LACTOSE, AND CALCIUM AND PHOSPHORUS UTILIZATION

The effect of lactose upon the absorption and utilization of calcium and phosphorus has been of considerable interest and a great deal of work has been done on this aspect of the over-all effects of lactose feeding. One of the first published reports on this aspect of lactose feeding was a report

by Bergeim *(12)*, who reported that lactose, even at a level of 25% of the diet, led to a pronounced increase in the amount of calcium and phosphorus absorbed, and was far superior in this respect to any other carbohydrate tried. It was believed that this increased absorption was due to increased lactic acid fermentation in the intestines, with the resulting increased acidity of the intestinal contents.

Greenwald and Gross *(41)*, in experiments with parathyroidectomized dogs, noted that lactose would protect such dogs against tetany if the diet contained large amounts of calcium. They believed that the lactose acted by making the calcium more available to the animal.

Robinson *et al. (84)*, in experiments with dairy calves, reported that the simultaneous feeding of lactose and bonemeal doubled the calcium retention from the bonemeal, but caused no change in the calcium retention from calcium lactate. Such feeding doubled the phosphorus retention from bonemeal and increased to about the same degree the retention of dietary phosphorus under the influence of calcium lactate.

Robinson and Duncan *(83)* reported that 25% lactose caused a marked lowering of the pH of the contents of the digestive tract below the duodenum. They believed that the effect of lactose on calcium absorption was due to this increased acidity of the intestinal tract.

Kline *et al. (53)* fed lactose to the young chicks at a level of 40% of a rachitic ration and reported that lactose had a favorable effect upon calcium absorption and upon ossification, as well as having a positive influence in maintaining a more acid condition in the entire intestinal tract. Lactose fed at a 20% level improved calcification to a smaller extent. This protective effect against rickets was also reported by Sunderlin *(94)*, who noted that lactose showed a definite prophylactic effect against rickets when 25% was added to a rachitic ration for rats.

French and Cowgill *(34)* reported that age might be a factor determining the favorable influence of lactose on the utilization of calcium and phosphorus. In their experiments with dogs, the data obtained with young animals indicated quite definitely that the presence of lactose in the diet favorably influenced the utilization of calcium and phosphorus. Such results were not obtained with mature dogs. Experiments with rats confirmed the dog experiments and indicated further that the degree of maturity plays a role in determining the effect of lactose on the utilization of calcium. These experiments with rats indicated that lactose acts to diminish the excretion of calcium into the intestine.

Outhouse *et al. (75)* made a comparative study of the growth-promoting and bone-calcifying effects of several carbohydrates. In paired feeding tests, no alteration in growth was observed when 25% of sucrose or lactose replaced an equivalent amount of starch in the basal ration of rats. Of the carbohydrates tested, lactose was the only one which caused an acceleration of bone calcification. In a later study by Outhouse *et al. (76)*, designed to study the relative effects of certain saccharides and vitamin D on the mineral metabolism

of rats, it was shown that lactose and cod liver oil rations caused greater retention of calcium, phosphorus, and magnesium than did rations containing starch or sucrose. Greater increments in retention, however, were obtained in the cod liver oil-fed animals than in the lactose-fed animals.

Mills *et al. (65)* studied the influence of lactose on calcium retention in children. Five boys ranging in age from five to seven years were studied for an experimental period of 16 weeks. They were fed a basal dietary to which $CaHPO_4$ was added, in order to raise the level of calcium intake to approximately 500 mg. daily. For from six to nine consecutive weeks they were also given a supplement of lactose (36 g. daily). When calcium balances were determined for the nonlactose and lactose periods, it was found that lactose caused a retention of calcium over the nonlactose period which amounted to an average of 33.5% for the five children.

Roberts and Christman *(82)* presented evidence which indicated that lactose did not aid in the absorption of calcium in the fasting rat given a measured dose of calcium lactate and killed three hours later for analysis and measurement of calcium absorption.

Fournier *et al. (33)* and Fournier *(29)* presented further evidence that lactose increased the absorption and utilization of calcium. These workers believed that the beneficial effects of lactose on ossification are more direct than by simply favoring the acidification of the intestinal environment. Additional information by Fournier *et al. (31, 32)* on the effect of various sugars in increasing the utilization of calcium indicated that, of the sugars tested, only lactose, galactose, xylose, and arabinose were capable of increasing calcium utilization. These authors also presented evidence to show that the sugars which exert the greatest effect on calcium utilization are those which are attacked to a lesser extent by the intestinal bacteria. They reported that since L-xylose was not attacked by the intestinal flora, it was very effective with regard to the utilization of calcium, although it could not act on calcification in a direct metabolic way. They postulated that those sugars which improved calcification did so by providing a favorable metabolism peculiar to ossification.

LACTOSE IN HUMAN FEEDING

The possible use of lactose in human nutrition, except for the infant or young child, has not been studied extensively. Kopeloff and Cheney *(57)* reported that normal and psychotic patients had obtained relief from chronic constipation and diarrhea by the ingestion of lactose (from 100 to 300 g.). Henneberg *(47)* reported that the presence of milk or milk products unquestionably acts to change the intestinal flora. Lactic acid bacteria were increased, whereas coli, putrefactive, and spore-forming bacteria were decreased. This was especially true with reform yoghurt. Maintenance of the desired flora depended upon reinoculation and a constant nutrient supply in the form of milk or milk sugar.

A study of the nutritive value of lactose in man was made by Koehler *et al. (56)*. They noted that the average blood sugar values in 16 normal young

adults after ingestion of 1.5 g. of lactose per kilogram of body weight showed no important changes, although considerable individual variations were noted. The secondary hypoglycemic effects were lacking and a lactosuria was frequently present. They also reported that the blood sugar changes after lactose ingestion by the diabetic were marked, and of nearly the same magnitude as after glucose ingestion. In the diabetic, however, lactosuria was never noted. Moore *et al. (71)* followed the growth and nutritional status of 144 children for approximately one and one-half school years. Half of these children received nonfat dry skimmilk in the school lunch, the average daily consumption being equivalent to 9.1 fl. oz. of milk. The differences in gains between the paired children were of a small order, but consistently in favor of the group receiving the milk solids. These workers reported that the continued and increased use of dry skimmilk solids in school lunches seemed practical and worth while.

LACTOSE IN INFANT FEEDING

It would appear that lactose being one of the normal nutrients of the infant, would find wide use in infant feeding. By the use of lactose, cow's milk could be made to resemble human milk quite closely. Considerable work has been done along this line, particularly in the formulation of synthetic mixtures for nursing infants.

Pehu and Porcher *(77)* indicated that lactose may be used in various amounts in the food of infants. From 18 to 36 g. may be added to the daily food intake without changing the taste of the food. Lactose was reported to be especially useful in the preparation of cow's milk, and to act not only as a food but also as a nonirritating laxative. Klinke and Konig *(54)* reported that they had obtained excellent results with lactose-enriched milk. They further reported that lactose had a particularly excellent influence on the turgor and condition of the skin, condition of the stool, resistance against infection, and disposition of the infants, and had a favorable effect on the increase in weight, and growth in length, of infants.

The influence of lactose on the metabolism of infants was studied by Talbot and Hill *(95)*, who reported that up to a certain level lactose tended to affect favorably the absorption of fat and the retention of nitrogen and ash. Beyond such level there was a greatly increased acidity of the stool, accompanied by diarrhea, and an unfavorable effect on the retention of nitrogen and ash and on the absorption of fat. They also reported that the amount of lactose which an infant could digest and absorb varied with the individual.

Porter *et al. (78)* reported that certain nutritional disorders of children, associated with a putrefactive intestinal flora, could be treated with lactose and dextrins, which are the carbohydrates most effective in encouraging the rapid establishment of a fermentative-type flora in the intestines of infants and children.

Gerstley *et al. (36-39)*, in rather extensive studies on the influence of feeding on certain acids in the feces of infants reported that in babies breast-fed and later changed to cow's milk, the volatile acid output and the total titratable

acidity were relatively constant when breast milk was fed and extremely variable when cow's milk was fed. It was also apparent that the stools of infants fed on cow's milk were much heavier than those of infants fed on breast milk. Changing from breast milk to cow's milk invariably caused the appearance of propionic acid in the feces. These workers also reported that the addition of an excess of lactose to breast milk caused little change in the excretion of the volatile or titratable acids, or in the weight of the stools; whereas, such addition of excess lactose to mixtures of modified cow's milk caused only a slight change in the total excretion of the volatile acids, but did increase the amount of free acid. The addition of 12% lactose to cow's milk created a relationship of total titratable to total volatile acidity resembling that found when breast milk was fed. They further reported that when the diet of an infant on breast milk was changed to whole cow's milk, formic acid disappeared from the stool, acetic acid predominated, and propionic acid appeared. Twelve per cent lactose often produced great changes in the stools, reducing the weight to approximately that of breast-fed infants' stools. From the reports of these workers, it would appear that the addition of 12% lactose to cow's milk would produce a food for infants which closely resembled breast milk.

Barenberg and Abramson *(6)* studied the effect of large amounts of milk sugar on the stools and nutrition of infants. They reported that the ingestion of 12 to 15% did not bring about loose stools. Sugar was not found in the urine or stools, despite the fact that the infants received approximately 15 to 17 g. of lactose per kilogram of body weight. These workers concluded that lactose was a safe carbohydrate for the modification of cow's milk, and that it did not have a laxative effect in infants, even when used in high concentrations.

Hardisty *(45)* presented an excellent review of the literature on infant feeding and concluded that lactose was the sugar of choice for infant feeding. Powers *(79)* presented some historical background on infant feeding and gave some rules for the formulation of infant diets, using lactose as the carbohydrate.

The influence of beta-lactose on the intestinal flora of infants was studied by Primnig and Turkus *(80)*, who reported excellent results and recommended its use in infant feeding, since with its use the best possible microfloral environment would prevail and dangerous intestinal infections would be kept to a minimum. Acker *(1)* made a study of the importance of alpha- and beta-lactose in the nutrition of children and reported that breast milk contained preponderantly the beta form, which was regarded as the more beneficial nutritionally. The change from natural to artificial feeding resulted in a shift of the pH from faintly acid to faintly alkaline, and caused a replacement of the bifidum flora with a mixed flora. This same change in the intestinal flora was observed after a change from beta- to alpha-lactose. Frisell *(35)* showed that both alpha- and beta-lactose, administered by mouth, could produce in certain cases a moderate increase in the bifidum count in the feces of

healthy infants. No difference was found between alpha- and beta-lactose with respect to bididum-stimulating capacity, nor did any unequivocal changes occur in certain other fecal flora or in the physical characteristics of the stools during the period in which lactose was given.

According to Jarvis *(50)*, the lactose-fed infant resembles but does not equal the breast-fed infant in firmness of tissues and resistance toward infections. Age-weight for age-weight, the lactose-fed infant was shown to possess more living tissue than the infant fed on vegetable sugar. This was due to the lower water content, as well as the lower fat content, of the tissues of infants fed on milk sugar. Lactose formulas facilitated complementary and supplementary feedings with less danger of refusal by either the breast-fed or bottle-fed infant, and lessened the danger of the development of habitual anorexia or constipation. As a source of galactose for the development of the central nervous system, lactose was thought to be especially desirable as a sugar for modified feeding in an optimal amount. Delthil *(21)* observed that in infants fed lactose the intestinal flora resembled that of the breast-fed infant, the mineral assimilation appeared to be better, and the myelinization of nerve fibers was more rapid. He also reported that lactose could be used in the feeding of premature infants, because the addition of 2% of lactose to human milk for such premature infants would favor the development of the nerve fibers of the central nervous system.

MISCELLANEOUS

Maier and Longhurst *(62)* studied the effect of a lactose-free diet on the problem-solving behavior in rats. From their results, they concluded that the lactose diet did not create a deficiency in performance, but that the removal of lactose from this same diet did produce a deficiency in performance, as shown by three of the four groups reared and maintained on such diets. Analysis of results of rats on lactose-free diets indicated that the reduced performance could be attributed to reduced ability. They concluded that lactose had a nutritive value other carbohydrates did not have.

Cairns *(14)*, in an article on lactose production and utilization in Australia, lists the following as the main uses of lactose in Australia: in infant and proprietary foods, for modifying cow's milk for infant feeding, in medicine as a laxative and diuretic, for coating pills, as a medium for penicillin culture, as a reducing agent in the making of mirrors, and in the manufacture of some smoke-bombs and flares.

The effect of alloxan in rats fed corn starch, sucrose, and lactose was studied by Leonard *et al. (61)*. The highest susceptibility was observed for rats on the corn starch diet. There was a slightly decreased susceptibility on the sucrose diet, whereas the lowest susceptibility was observed on the lactose diet.

Fournier *(30)* reported that lactose had a lipotropic action similar to choline, and postulated that this common effect between choline and lactose was owing to the fact they both could furnish glycine to the animal. Sadhu *(88)*, in reporting on lactose as a lipotropic agent, made the observation that the

lactose-fed rats had greater choline and phospholipid and less fat and sphingomyelin content in the liver and kidney. This experiment indicated that lactose acted not only a a sparer of choline but also as a lipotropic agent. This was related to the Kwashiorkor syndrome, since the lack of milk and the absence of lactose in the diet of the young after early weaning could be responsible for the fatty liver, as a result of the lack of the choline-sparing and lipotropic action of lactose.

Whitnah and Bogart *(98)* studied the reproductive capacity of female rats as affected by the kind of carbohydrate in the ration, and reported that the sucrose-containing ration was inadequate for normal reproduction in rats, even when replaced during adult life by normal action. These animals showed a precocious sexual development. Animals fed diets containing lactose or cornstarch reproduced normally and had normal ovarian structures.

Harper *et al. (46)* studied the influence of dietary carbohydrate on the level of amino acids in the feces of rats, and reported that the inclusion of 10% lactose in a ration containing sucrose as the source of carbohydrate caused a statistically significant increase in the levels of lysine and histidine in the feces. They postulated that the influence of lactose might be related to changes in the intestinal flora.

DISCUSSION

In this review it has been pointed out that young mammals are able to tolerate large quantities of lactose, but that this tolerance becomes less as the animals become older. Since lactose is one of the natural foods of the young mammal, this is understandable, and indicates that lactose could be widely used in animal feeding, particularly during the early stages of growth. This is especially true for the young pig or young dairy calf.

Poultry do not tolerate even moderate levels of lactose, regardless of the age. This could probably be expected, since lactose is not a natural food of poultry.

Lactose cataract in rats fed high levels of lactose has been described many times by many different workers, yet the cause of this phenomenon is still much a mystery. It appears that additional work should be done on this problem, with an effort made to determine just what are the causes of this phenomenon, since there might exist a relationship between this and other types of cataract.

The work of Maier and Longhurst *(62)* is very interesting and could be important. More work along this line appears to be warranted. This is also true of the report by Whitnah and Bogart *(98)*, where additional work also might be worth while.

Already much work has been done on the value and uses of lactose in animal and human feeding, but additional work could well be done in almost all phases and aspects of the problem of using lactose in all types of animal and human foods.

SUMMARY

An attempt has been made to review the available information on the uses of lactose in animal and human feeding. From the information reviewed, the following conclusions can be drawn:

1. Lactose is tolerated by the rat up to about 25% of the diet. Above this level poor growth, diarrhea, alopecia, and cataract formation result.

2. Young pigs and dairy calves are able to tolerate extremely high levels of lactose. This tolerance for lactose decreases with the age of the animal.

3. Poultry, either young or old, are unable to utilize even moderate levels of lactose.

4. Lactose has a definite effect on the intestinal tract of all animals. This effect is characterized by a lowering of the acidity and a change of the intestinal flora to an acidophilic type.

5. Lactose stimulates the synthesis of B-vitamins by the intestinal bacteria in both mammals and poultry.

6. Lactose feeding causes a change in the phospholipids of the tissues of animals fed diets containing large amounts of lactose.

7. Lactose favorably influences the absorption, retention, and utilization of calcium, phosphorus, and magnesium. This may or may not be related to the change in pH of the intestinal tract following lactose feeding.

8. Lactose is well-tolerated by the human adult and it may be used in therapy against constipation and diarrhea.

9. Lactose finds wide use in infant feeding and should be considered the carbohydrate of choice for the modification of cow's milk and for the formulation of infant foods. It is especially useful as an additive to human milk for the feeding of premature infants.

10. Lactose has been shown to improve the problem-solving ability of rats, which suggests a nutritive value not found in other carbohydrates.

11. Lactose has been shown to protect the rat against alloxan poisoning. Sucrose and starch did not give this protecting action against alloxan poisoning.

12. Lactose has been shown to be a lipotropic agent and a sparer of choline.

13. Lactose allows normal reproduction in the rat. This was not true for sucrose.

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DESIGN AND CONDUCT OF CALF NUTRITION STUDIES. 1. ONE- *VS.* TWO- AND THREE-DAY GROWTH MEASUREMENTS[1]

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Effects of 1-, 2-, and 3-day measurements for weight, heart girth, girth at paunch, height at withers, and length from withers to pin bones for 48 calves, raised on a limited whole milk–calf starter system, were studied at biweekly intervals for 13 weeks. Because the components of variance approach for studying different numbers of measurement was found to be questionable, measurements taken on successive days on the same calf can not be safely assumed to be independent; conclusions were drawn from the errors estimated by using the 1-day measurements or the average of 1 and 2 or the average of 3 and making comparisons among these errors by the Pitman-Morgan test for correlated mean squares. A single-day measurement was sufficient to determine growth in weight, heart girth, and girth at paunch; whereas, 3-day measurements were significantly better than 1- or 2-day measurements for length from withers to pin bones, but the results were inconclusive for height at withers. Sizes of the coefficients of variation for each growth criteria decreased markedly up to 6 weeks, and thereafter the decreases were relatively small. *Editor.*

Physical measurements are used in nutrition experiments to determine the effect of different rations or nutrients on growth. This effect is assessed by a comparison of the changes with time in measurements, ordinarily the differences between initial and final measurements, of animals on different treatments. This comparison is subject to two kinds of error: *(a)* between-animal variability in growth, which is present even when animals of the same age, sex, and breed are used, and *(b)* measurement variability. These constitute the error or residual variability found in the analysis of variance. By taking an infinite number of determinations of a measurement on each of the experimental animals, it is theoretically possible to eliminate the measurement variability entirely. If, however, the animal variability is much greater than the measurement vari-

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ability, eliminating the measurement variability entirely will make little difference in the total experimental error. In such a case, a single determination of a given measurement will be as good as the average of many such determinations.

In studies on the errors in the weighing of animals, Lush and Black *(12)* and Lush *et al. (13)* concluded that three-day average live weights had greater precision than one- or two-day averages. Inappreciable gain in precision was obtained by increasing the number of weighings beyond three. Bean, working with swine *(3)*, and Baker *et al.* with beef calves *(2)*, found smaller variations, animal plus measurement variability, in first- than in second- or third-day weights or in the average of these three-day weights. As has been pointed out recently by Touchberry and Lush *(20)*, grouping the animals on the basis of the first-day weights in the latter two studies accounted for at least a part of the higher variance in the second- and third-day weighings, as well as in the average of the three-day weights. No reduction in the animal plus measurement variability by use of a three-day instead of a single determination of initial and final live weights was reported by Bean *(4)* for lambs. Working with steers, Patterson *(18)* reported that, by increasing the number of weighings from one to three, variation in the absolute weights was reduced less than 2.5%, and variation in the gain in weight was reduced less than 8%.

Studies on the variability of linear measurements have been reported by Lush *et al. (14)*. Twenty-five different linear measurements, taken 11 times on each of nine Jersey cows and ten heifers, indicated that, in most cases, single measurements were precise enough to obtain significant differences between animals. Similar findings with dairy cattle at each of seven age intervals for height at withers, chest depth, heart girth, and paunch girth were reported by Touchberry and Lush *(20)*. Greater precision was achieved by increasing the number of measurements from one to three for body length.

The objective of the present study was to compare the effect of one-, two-, and three-day measurements on the experimental errors in growth studies with dairy calves. These comparisons were made for growth in terms of live weight, heart girth, girth of paunch, height at withers, and length from withers to pin bones.

EXPERIMENTAL PROCEDURE

Animals and rations. Forty-eight calves—24 Guernseys and 24 Holsteins—balanced within each breed group with respect to sex, were placed on experiment at one day of age. The Guernseys were from the herds of the University of Connecticut, and the Lyman Farm, Middlefield, Conn.; the Holsteins from the herds of the University of Connecticut, the Mansfield State Training School and Hospital, Mansfield Depot, Conn., and the Osborn Prison Farm, Hazardville, Conn. Half of the calves, balanced with respect to breed and sex, were placed on experiment during September–December, 1951, and the other half during August–September, 1952. The calves were separated from their dams shortly after birth, transported to the University research barn, and there placed in individual tie-stalls, in a separate portion of the barn in which the tem-

perature was never permitted to become less than 10° C. The position of the calves in the barn was balanced with respect to breed and sex, in groups of three.

For a 24-hr. period each calf was allowed either to nurse its dam or was fed colostrum from its dam or from a frozen colostrum bank, at the daily rate of 7.0 lb. for Guernseys and 8.0 lb. for Holsteins. Thereafter, Holstein herd milk was fed to 35 days of age, on the basis of weekly live weights adjusted to the 0.7 power, as previously described *(8)*. Milk was fed twice daily at 7 A.M. and 5 P.M. to the 21st day of age, and only in the mornings thereafter. From the eighth day of age on, water and hay were allowed ad lib., and starter up to a daily maximum of 2.5 lb. for Guernseys and 3.0 lb. for Holsteins. Starter was fed once daily between 7 and 9 A.M. Hay and water were replenished, when necessary, between 7 and 9 A.M. and 3 and 4 P.M.

The calf starter consisted of standard ingredients[4] and was pelleted into 3/16-in.-diameter pellets. The hay, which was chopped to 1/4-in. in a portable chopper, graded on the average U. S. No. 1.6 and U. S. No. 1 Green Mixed, the first and second years, respectively. Its average botanical composition, for the first and second years, respectively, was 11.7 and 3.2% foreign material, with the remainder made up of 44.8 and 31.2% clovers, 0.4 and 7.4% alfalfa, and 54.8 and 61.4% grasses. The average chemical compositions of these feeds are shown (Table 1).

Diarrhea, when accompanied by rectal temperatures of 103° F. or above, was treated according to standard procedures followed at this Experiment Station *(8)*.

Observations. Feed intakes and refusals were weighed to the nearest 0.1 lb. Refusals of starter and of hay were weighed prior to feeding on the fourth and eight mornings of each calf's successive seven-day age periods. There were no milk refusals.

Live weight was measured to the nearest 1.0 lb. with a Toledo platform scale (Model 31:1511fc); height at withers to the nearest 1/4-in. with a wooden caliper, with a spirit level in the center of the horizontal arm; heart girth, girth of paunch, and length from withers to pin bones to the nearest 1/4-in. with a steel tape (Lufkin No. C2100). These measurements were similar to those diagrammed by Brody *(5)*, with the exception of length from withers to pin bones, which was essentially a combination of two of Brody's measurements.

Each calf, halter-led, was weighed and measured on three successive days at weekly intervals, beginning on the sixth, seventh, and eighth, and ending on the 90th, 91st, and 92nd days of age. For purposes of this study, only

[4] The starter was composed of the following ingredients in pounds per ton of mixture: 409.5 ground corn, 400 ground oats, 300 wheat bran, 140 linseed oil meal, o.p., 280 soybean oil meal, o.p., 200 artificially dehydrated alfalfa meal (guaranteed potency of 100,000 U.S.P. units of vitamin A per lb.), 100 dried skimmilk, 40 B-Y 500-potency dried fermentation solubles, 20 dicalcium phosphate, 10 iodized salt, 100 cane molasses, and 0.5 irradiated yeast (Standard Brands Type 36-F with guaranteed potency of 16 million I.U. of vitamin D per lb.). During the second year, 1952, because of the inability of obtaining readily the B-Y 500-potency dried fermentation solubles, 10 lb. of Fortafeed (Lederle's 2-22C) were used, and the corn meal was increased by 30 lb.

TABLE 1

Average chemical composition of the feeds with their standard errors

Feed	Composition						
	Dry matter	Crude protein	Ether extract	Crude fiber	Nitrogen-free extract	Ash	Carotenoids
	(%)	*(% of the dry matter)*					*(mg/lb)*
First year							
Mixed hay							
As fed[a]	91.56 ± 0.66	10.69 ± 0.21	1.97 ± 0.09	32.07 ± 0.37	47.75 ± 0.17	7.52 ± 0.20	4.74 ± 0.30
As refused[a]	91.53 ± 0.73	11.49 ± 0.33	2.16 ± 0.14	31.08 ± 0.51	47.72 ± 0.44	7.55 ± 0.17	4.68 ± 0.24
Starter[a]	91.66 ± 0.56	22.63 ± 0.12	3.63 ± 0.13	9.19 ± 0.10	57.09 ± 0.20	7.46 ± 0.07	4.52 ± 0.34
Second year							
Mixed hay							
As fed[b]	91.16 ± 0.57	12.45 ± 0.93	3.29 ± 0.70	29.82 ± 0.61	47.74 ± 1.69	6.70 ± 0.60	7.92 ± 1.19
As refused[c]	91.84 ± 0.20	11.88 ± 0.67	3.03 ± 0.87	29.72 ± 0.49	48.65 ± 0.90	6.72 ± 0.28	6.87 ± 1.47
Starter[b]	90.81 ± 0.25	21.47 ± 0.13	4.77 ± 0.45	7.68 ± 0.09	58.86 ± 0.84	7.22 ± 0.49	3.35 ± 0.62

[a] Average of eight samples.
[b] Average of five samples.
[c] Average of four samples.

biweekly measurements were used. Weights and measurements were taken between 1 and 3 P.M. daily. Single determinations of live weight and length from withers to pin bones and the average of three determinations, rounded to the nearest 1/4-in., of height at withers, heart girth, and girth of paunch were recorded. Separate data sheets were used for each day and these were not referred to on subsequent days. Since the same person could not be present at all times, three persons similarly trained, performed the weighing and measuring. Assignment was according to availability and was in no way balanced as to individuals. Average values of live weight and of the linear measurements are presented (Appendix Table 1).

Representative material fed and refused was collected at successive 28-day intervals for proximate analyses by standard procedures *(1)*. Carotenoid content was determined by the method of Moore and Ely *(16)*, modified by using a mixture of starch-supercel as the chromatographic absorbent.

Days with scours were recorded from observations made three times daily, from 5 to 9 A.M., 1 to 3 P.M., and 5 to 6 P.M.

Statistical analysis. Since, in nutrition experiments, ration effects are generally assessed by the change in a measurement (growth) over a period of time, the statistical studies were devoted to estimating the errors with which this change (growth) is measured. Growth periods of two weeks, four weeks, ... , 12 weeks duration were studied, the initial measurements being for one week of age in all cases. Growth was based on one-day initial and one-day

final measurements, two-day average initial and two-day average final measurements, and three-day average initial and three-day average final measurements. All five growth criteria were handled in the same way.

Two procedures were employed for estimating the error: a "direct" estimation procedure and the "component" estimation procedure. Two variations of the component approach were used.

In the direct approach, the first of the three consecutive initial and of the three consecutive final measurements were used to compute growth based on one-day measurements. For growth based on two-day average measurements, the first two days of the three-day sets were used. Finally, growths were computed using the average of all three initial and all three final measurements. For each growth period and each growth criterion, an analysis of variance was done according to the following scheme:

Source of variation	*Degrees of freedom*
Subgroups (breed, sex, year, and their interactions)	7
Calves within subgroups (error)	40
Total	47

A reviewer of this paper noted that the one-, two-, and three-day results obtained by the direct approach may not be comparable, because the actual period of observation was one day longer in the two-day results than in the one-day results, and two days longer in the three-day results. To examine this question, a modified direct approach was studied for two- four-, and six-week growth periods. The modification consisted of using the last instead of the first of the three consecutive final measurements to compute one-day results, and the last two days instead of the first two days of the three final measurements to obtain the two-day results. In order to convert the growths so obtained to the proper scale for comparison, the one-day values were multiplied by $n/(n+2)$, and the two-day values by $n/(n+1)$, where n is the number of days between the first initial and first final measurement.

Comparisons of the error mean square obtained for growths based on one-, two-, and three-day initial and final measurements were made with the Pitman-Morgan test *(17, 19)* as given by Cochran *(6)*. This test for comparing correlated mean squares was necessary, because the growths using one-day initial and one-day final measurements were based on data which were employed also to compute growths based on two- and three-day average measurements.

In the component approach, the error mean squares were considered to be divisible into three components. These components were, *(a)* that due to variability in growth among calves within subgroups (σ^2_c), *(b)* that due to variability among the three initial measurements on the same calf (σ^2_i), and *(c)* that due to variability among the three final measurements on the same calf (σ^2_f). The error mean square when using n-day measurements ($n = 1$, 2, or 3) thus was expressed in terms of these components, as follows:

$$(EMS_n) = \sigma^2_c + \sigma^2_i/n + \sigma^2_f/n$$

Note that when $n = 3$, the error mean square (EMS_3) is given directly by the error mean squares obtained by the direct approach for growths based on three-day measurements. The components σ^2_i and σ^2_f were estimated by analyzing each three-day set of measurements separately, according to the following analysis of variance:

Source of variation	*Degrees of freedom*
Subgroups	7
Calves within subgroups	40
Days	2
Linear trend	1
Residual	1
Days by subgroups	14
Linear trend by subgroups	7
Residual by subgroups	7
Days by calves within subgroups	80
Linear trend by calves within subgroups	40
Residual by calves within subgroups	40
Total	143

In the first variation of the component approach, the mean squares for days by calves within subgroups provided estimates of σ^2_i and σ^2_f. In the second variation, the mean squares for residual by calves within subgroups were used as estimates of σ^2_i and σ^2_f. Thus, in the latter approach, the linear growth-trend specific to each three-day set of measurements on each animal was eliminated.

In the first variation of the component approach, σ^2_c was estimated by substituting the estimates of σ^2_i and σ^2_f obtained from the day-by-calf within subgroups interaction into the relation:

$$\sigma^2_c = EMS_3 - \sigma^2_i/3 - \sigma^2_f/3^5$$

In the second variation, σ^2_c was estimated by substituting σ^2_i and σ^2_f obtained from the residual by calves within subgroups mean square. Estimates of the error mean squares to be expected for growths based on one- or two-day average measurements were then obtained by substituting the appropriate values of the three components into the relationship:

$$\text{Est } (EMS_n) = \sigma^2_c + \sigma^2_i/n + \sigma^2_f/n^5$$

and letting n take the value of 1 or 2, as required.

RESULTS AND DISCUSSION

The direct vs. the component approach for estimating the error mean squares. The error mean squares (calves within subgroups) for the single determination and for the two- and three-day average determinations, as obtained by the direct procedure and by both variations of the component procedure,

[5] These are estimates of σ^2 rather than true values.

are given (Table 2). The error mean squares derived by the two component methods showed, in general, a considerably greater reduction in variability due to increasing the number of measurement days from one to three than was shown by the direct procedure.

In both variations of the component method, the assumption is made that a measurement taken on a given day is not affected by whether or not the animal has been measured previously. In the first variation of the component approach, it is assumed that no time-trend exists over the set of measurements taken on three consecutive days. In the second variation of the component method, the assumption is made that there exists a linear time-trend over the three consecutive days. In other words, in the component approach it is assumed that the observations are independent after correction for the mean over the three days and, in the second variation, after correction also for linear time-trends.

In contrast, the direct approach involves no assumptions about the independence of the successive observations. It would be expected to agree with one or the other of the component approaches only if the observations on successive days were independent. Results show that the successive measurements safely cannot be assumed to be independent; therefore, that the component approach is subject to question. An attempt was made to modify the components approach by assuming that correlation existed between measurements made on successive days. This attempt was unsuccessful because the authors were unable to obtain a number of independent equations sufficient to estimate all components of variance and covariance. Because the component approach is subject to serious doubt, the ensuing discussion will be based almost entirely on the results of the direct approach.

Effect of increasing the number of measurement days. Ratios of the error mean squares for one- to two-, one- to three-, and two- to three-day average determinations are given (Table 3). A ratio greater than one indicates that the error mean squares were reduced by increasing the number of measurement days; a ratio less than one indicates that the error increased with increasing measurement days. The respective student's t values are also shown, to aid in assessing the significance of the comparisons.

For live weight, some increase in the size of the error mean squares was observed with an increase in the number of measurements. However, for single- *vs.* two-day values, none of the increases was statistically significant.[6] Two of the increases were significant for single- *vs.* three-day values, and three for two- *vs.* three-day values.

The error mean squares for heart girth and girth of paunch were generally smaller for two- or three-day measurements than for one-day measurements, but few of the decreases were significant. In general, there was little difference

[6] The two-tailed test is used to test the hypothesis $MS_1 = MS_2$ with the alternate hypothesis $MS_1 \neq MS_2$; the one-tailed test, to test the hypothesis $MS_1 = MS_2$ with the alternative hypothesis $MS_1 > MS_2$ or $MS_1 < MS_2$.

TABLE 2

Error mean squares for growth, based on single-, two-, and three-day initial and final measurements, as estimated by the direct and by the component procedures

Growth criterion and basis of measurement	Growth period (days of age at first initial and first final measurements)					
	6 to 20	6 to 34	6 to 48	6 to 62	6 to 76	6 to 90
	Error mean squares as estimated by the direct approach					
Live weight *(lb.)*						
1-day	6.86 (5.86)[b]	28.74 (31.44)	38.93 (37.81)	59.46	83.19	110.24
2-day	5.96 (5.66)	29.97 (30.69)	39.26 (37.77)	59.75	84.45	110.51
3-day[a]	7.37	33.22	41.01	58.80	90.55	112.08
Heart girth *(in.)*						
1-day	0.4922(0.1457)	0.3029(0.4022)	0.3943(0.4460)	0.5776	0.6448	0.7289
2-day	0.2852(0.1378)	0.2534(0.2706)	0.3117(0.3102)	0.4295	0.5344	0.5551
3-day	0.1818	0.2674	0.3243	0.4118	0.5427	0.5670
Girth of paunch *(in.)*						
1-day	1.0628(1.0968)	2.9125(2.0623)	1.8792(1.6559)	2.6987	3.0622	3.4870
2-day	0.8076(0.9107)	2.4853(2.0786)	1.7167(1.3968)	2.7389	3.1567	3.1405
3-day	0.9512	2.6037	1.5177	2.6118	3.5239	3.1478
Height at withers *(in.)*						
1-day	0.1206(0.1033)	0.1883(0.2128)	0.2289(0.2019)	0.2531	0.2641	0.3010
2-day	0.0728(0.0783)	0.1527(0.1561)	0.1938(0.1968)	0.2346	0.2617	0.2461
3-day	0.0662	0.1449	0.1716	0.2043	0.2223	0.2131
Length from withers to pin bones *(in.)*						
1-day	0.6076(0.6450)	0.6990(0.5548)	0.5977(0.8378)	1.0021	1.4500	0.7818
2-day	0.3771(0.4004)	0.3438(0.3578)	0.3371(0.4120)	0.5860	0.8710	0.7702
3-day	0.3083	0.2912	0.3073	0.4663	0.6155	0.6385
	Error mean squares as estimated by the component approach, disregarding time-trend					
Live weight *(lb.)*						
1-day	8.89	35.07	42.61	60.63	92.23	114.44
2-day	7.75	33.68	41.41	59.25	90.97	112.67
3-day[a]	7.37	33.22	41.01	58.80	90.55	112.08
Heart girth *(in.)*						
1-day	0.3613	0.4383	0.5041	0.6456	0.7688	0.8157
2-day	0.2267	0.3101	0.3692	0.4702	0.5992	0.6291
3-day	0.1818	0.2674	0.3243	0.4118	0.5427	0.5670

Girth of paunch *(in.)*						
1-day	1.4150	3.1002	2.1861	3.4652	4.1353	3.8319
2-day	1.0671	2.7279	1.6848	2.8251	3.6768	3.3188
3-day	0.9512	2.6037	1.5177	2.6118	3.5239	3.1478
Height at withers *(in.)*						
1-day	0.1484	0.2143	0.2441	0.2993	0.3117	0.2972
2-day	0.0866	0.1621	0.1897	0.2280	0.2445	0.2340
3-day	0.0662	0.1449	0.1716	0.2043	0.2223	0.2131
Length from withers to pin bones *(in.)*						
1-day	0.6115	0.6083	0.6997	0.8854	1.1204	1.1368
2-day	0.3841	0.3705	0.4053	0.5710	0.7416	0.7630
3-day	0.3083	0.2912	0.3073	0.4663	0.6155	0.6385
Error mean squares as estimated by the component approach, with adjustment for time-trend						
Live weight *(lb.)*						
1-day	8.38	34.23	41.90	60.13	91.65	113.63
2-day	7.62	33.47	41.23	59.13	90.82	112.46
3-day[a]	7.37	33.22	41.01	58.80	90.55	112.08
Heart girth *(in.)*						
1-day	0.3607	0.4559	0.5158	0.6461	0.7664	0.7835
2-day	0.2265	0.3145	0.3721	0.4703	0.5986	0.6211
3-day	0.1818	0.2674	0.3243	0.4118	0.5427	0.5670
Girth of paunch *(in.)*						
1-day	1.2808	2.9999	2.0576	3.4271	3.9536	3.8143
2-day	1.0336	2.7027	1.6527	2.8157	3.6314	3.3145
3-day	0.9512	2.6037	1.5177	2.6118	3.5239	3.1478
Height at withers *(in.)*						
1-day	0.1645	0.2261	0.2547	0.3134	0.3280	0.3060
2-day	0.0908	0.1652	0.1924	0.2316	0.2488	0.2363
3-day	0.0662	0.1449	0.1716	0.2043	0.2223	0.2131
Length from withers to pin bones *(in.)*						
1-day	0.5216	0.5252	0.5701	0.8493	0.9587	0.9813
2-day	0.3616	0.3497	0.3730	0.5621	0.7013	0.7242
3-day	0.3083	0.2912	0.3073	0.4663	0.6155	0.6385

[a] The three-day error mean squares are identical for all three methods, because they were all computed from the three-day means.
[b] Results obtained with the modified direct approach.

between two- and three-day measurements. For the six to 20 day-of-age growth interval, the use of the average of three successive day values for heart girth was significantly better than the use of single- or two-day values, and for the six- to 90-day interval, the use of the average of two-day values was superior to the use of single values.

The error mean squares for height at withers and length from withers to pin bones were consistently reduced by increasing the number of measurement days from one to three. For length from withers to pin bones, most of the reductions were statistically significant. In contrast, reductions for height at withers were rather inconclusive, in that statistical significance occurred at only three of the six age intervals studied.

The results obtained with the modified direct approach were essentially the same as with the direct approach.

Clearly, a one-day measurement is sufficient for estimating increases in live weight of the dairy calf. This agrees with the conclusions of Baker *et al.* *(2)* and Patterson *(18)* for cattle, and of Bean *(3, 4)* for swine and sheep. The studies of Lush and Black *(12)* and Lush *et al.* *(13)* were devoted only to measurement variability in weighing. Hence, they cannot be directly compared with this study or with the other studies cited which consider a combination of animal and measurement variability.

The data appear to support the contention of Bean *(3, 4)* and Baker *et al.* *(2)* that weighing for more than one day tends to increase the variability of live weights; however, the increases observed in this study do not conclusively support the view that single weights are more accurate than two- or three-day weights. Of the comparisons made, only five out of the 18 indicate statistically significant increases in the error mean squares associated with increased number of weighing days. Of these, the three from six to 20 and six to 34 days of age can possibly be explained by the smaller milk allowances on the third weighing day (see *Experimental Procedure*), which could have affected digestive fill in an erratic way.

No data pertaining to the effect of frequency of taking linear measurements, on the combined animal and measurement variability, were found in the literature. In this study (Appendix Table 2), the average size solely of the measurement errors, σ^2_i and σ^2_f, expressed as coefficients of variation was: 1.09 for heart girth, 1.80 for girth of paunch, 0.76 for height at withers, and 1.95 for length from withers to pin bones.[7] Except for height at withers, these values are slightly larger than those reported by Lush *et al.* *(14)* and by Touchberry and Lush *(20)*. A possible reason for this is that the calves were not as well-trained to the halter as were the older animals studied by Lush and coworkers. In the present investigation, no consistent relation of the size of measurement error to age of the calf was found. It should be noted that there is some question about the meaning of the measurement errors of this experi-

[7] These values represent the errors after the removal of the linear three-day trend. The values without the removal of the linear trend were 1.22, 1.96, 0.76, and 2.09, respectively (Appendix Table 1).

ment. They were estimated under the assumption, not safely tenable, that observations on successive days are not correlated.

Length from withers to pin bones showed, as compared to the other linear measurements, relatively large and consistent reductions in the error mean square as the number of measurement days was increased. This is associated with the fact that only one measurement per day was taken of the length from withers to pin bones; whereas, three observations per day were taken and averaged for the other linear measurements. It is reasonable to assume, nevertheless, that the withers to pin bones measurement is the least precise of the linear measurements, because the point of demarcation at the withers is subjective. This assumption is in agreement with the finding of Touchberry and Lush *(20)*, that length was the least precise of the linear measurements when the same number of measurements was taken per day.

Standard deviations, mean growth, and coefficients of variation. The errors previously discussed for one-, two-, and three-day average measurements are presented (Table 4) as standard deviations and coefficients of variation (standard deviation as a percentage of the mean growth). Also given are the corresponding mean growths. The standard deviations and coefficients of variation refer to the combined animal and measurement variability.

As the number of measurement days was increased, the mean growth for live weight, heart girth, and girth of paunch increased, as would be expected for young animals growing at an increasingly rapid rate. In contrast, the mean growth for height at withers appeared to decrease slightly, and that for length from withers to pin bones exhibited no consistent trend. Reflecting almost exactly the findings of the error mean squares, the standard deviations and coefficients of variation changed inappreciably for live weight, heart girth, and girth of paunch with an increase in the number of measurement days; whereas, those for height at withers, and especially those for length of withers to pins, decreased.

As the length of the growth period increased, the standard deviations consistently increased, but not so rapidly as did the growths. As a result, the coefficients of variation decreased. These decreases were more pronounced for the shorter growth periods (six weeks or less) than for the longer ones. The trends in the coefficients of variation for live weight are in excellent agreement with those reported in the classical study of Mitchell and Grindley *(15)*, for growing and fattening livestock and poultry.

The shorter growth periods in the present data were associated with the younger ages. As the length of the period increased, so did the average age during the period. To determine whether the trend in the coefficients of variation was associated primarily with age or primarily with length of growth period, coefficients of variation were computed for short growth periods at various ages. The periods used were six to 27, 27 to 48, 48 to 69, and 69 to 90 days of age. The coefficients of variation[8] were: for

[8] Live weight, heart girth, and girth of paunch based on one-day measurements; height at withers and length of withers to pin bones based on three-day measurements.

TABLE 3

Effect of frequency of measurement on the error mean squares (MS), in measuring growth by each of several criteria

Criterion	Growth period (days of age at first initial and first final measurements)					
	6 to 20	6 to 34	6 to 48	6 to 62	6 to 76	6 to 90
Live weight *(lb.)*						
1- *vs.* 2-day						
Ratio MS_1/MS_2	1.15 (1.04)[b]	0.96 (1.02)	0.99 (1.00)	1.00	0.99	1.00
t – value[a]	1.271 (0.360)	0.774 (0.428)	0.187 (0.000)	0.033	0.444	0.072
1- *vs.* 3-day						
Ratio MS_1/MS_3	0.93 (0.80)	0.86 (0.95)	0.95 (0.92)	1.01	0.92	0.98
t – value	1.534 (1.706*)	1.972* (0.650)	0.782 (1.186)	0.189	2.681*	0.376
2- *vs.* 3-day						
Ratio MS_2/MS_3	0.81 (0.77)	0.90 (0.92)	0.96 (0.92)	1.02	0.93	0.99
t – value	2.002* (3.078*)	2.105* (1.628)	1.017 (2.015*)	0.436	2.503	0.491
Heart girth *(in.)*						
1- *vs.* 2-day						
Ratio MS_1/MS_2	1.73 (1.06)	1.20 (1.49)	1.26 (1.44)	1.34	1.21	1.31
t – value	3.262* (0.231)	0.971 (2.194*)	1.490 (2.194*)	1.577	1.102	1.812*
1- *vs.* 3-day						
Ratio MS_1/MS_3	2.71 (0.80)	1.13 (1.50)	1.22 (1.38)	1.40	1.19	1.29
t – value	5.090* (0.869)	0.645 (1.904*)	0.990 (1.706*)	1.549	0.942	1.364
2- *vs.* 3-day						
Ratio MS_2/MS_3	1.57 (0.76)	0.95 (1.01)	0.96 (0.96)	1.04	0.98	0.98
t – value	3.526* (1.841*)	0.455 (0.107)	0.300 (0.483)	0.362	0.158	0.186
Girth of paunch *(in.)*						
1- *vs.* 2-day						
Ratio MS_1/MS_2	1.32 (1.20)	1.17 (0.99)	1.09 (1.18)	0.99	0.97	1.11
t – value	1.620 (1.396)	1.630 (0.062)	0.869 (1.046)	0.114	0.303	1.085
1- *vs.* 3-day						
Ratio MS_1/MS_3	1.12 (1.15)	1.12 (0.79)	1.24 (1.09)	1.03	0.87	1.11
t – value	0.502 (0.784)	0.850 (1.735*)	1.345 (0.424)	0.219	1.039	0.886
2- *vs.* 3-day						
Ratio MS_2/MS_3	0.85 (0.96)	0.95 (0.80)	1.13 (0.92)	1.05	0.90	1.00
t – value	1.339 (0.370)	0.606 (2.988*)	1.021 (0.766)	0.506	1.332	0.030

Height at withers *(in.)*						
1- *vs.* 2-day						
Ratio MS_1/MS_2	1.66 (1.32)	1.23 (1.36)	1.12 (1.02)	1.08	1.01	1.22
t – value	2.329* (1.448)	1.314 (1.928*)	1.050 (0.185)	0.426	0.058	1.200
1- *vs.* 3-day						
Ratio MS_1/MS_3	1.82 (1.56)	1.30 (1.47)	1.33 (1.18)	1.24	1.19	1.41
t – value	2.398* (2.138*)	1.497 (2.185*)	1.616 (1.007)	1.068	1.025	1.808*
2- *vs.* 3-day						
Ratio MS_2/MS_3	1.10 (1.18)	1.05 (1.08)	1.13 (1.15)	1.15	1.18	1.15
t – value	0.625 (1.213)	0.493 (0.872)	1.554 (1.396)	1.511	1.913*	1.698*
Length from withers to pin bones *(in.)*						
1- *vs.* 2-day						
Ratio MS_1/MS_2	1.61 (1.61)	2.03 (1.55)	1.77 (2.03)	1.71	1.66	1.02
t – value	2.602* (2.872*)	4.098* (2.826*)	3.070* (3.500*)	3.233*	3.673*	0.071
1- *vs.* 3-day						
Ratio MS_1/MS_3	1.97 (2.09)	2.40 (1.90)	1.95 (2.73)	2.15	2.33	1.22
t – value	2.951* (3.242*)	3.622* (3.007*)	2.577* (3.959*)	3.063*	4.941*	0.880
2- *vs.* 3-day						
Ratio MS_2/MS_3	1.22 (1.30)	1.18 (1.23)	1.10 (1.34)	1.26	1.12	1.21
t – value	1.505 (1.850*)	1.060 (1.343)	0.514 (1.103)	1.479	2.757	1.837*

* $P < 0.05$. At $P = 0.05$, $t = 1.684$ for the one-tailed test and 2.021 for the two-tailed test (40 d.f.).

[a] Pitman-Morgan test for correlated mean squares applied, Cochran *(6)*.

[b] Results obtained with the modified direct approach.

TABLE 4

Standard deviations for growth (S.D.), mean growth ($\overline{X}$), and coefficients of variation (C.V.) as affected by single-, two-, and three-day initial and final measurements, and by length of growth period

Growth criterion and basis of measurement	Growth period (days of age at first initial and first final measurements)					
	6 to 20	6 to 34	6 to 48	6 to 62	6 to 76	6 to 90
Live weight *(lb.)*						
Single measurement						
S.D.[a]	2.6 (2.4)[b]	5.4 (5.6)	6.2 (6.1)	7.7	9.1	10.5
$\overline{X}$	7.7 (8.5)	20.1 (20.9)	38.8 (39.9)	56.5	73.8	91.9
C.V.	34 (28)	27 (27)	16 (15)	14	12	11
2-day average						
S.D.	2.4 (2.4)	5.5 (5.5)	6.3 (6.1)	7.7	9.2	10.5
$\overline{X}$	7.8 (8.3)	20.6 (21.0)	39.5 (40.1)	57.0	74.3	92.3
C.V.	31 (29)	27 (26)	16 (15)	14	12	11
3-day average						
S.D.	2.7	5.8	6.4	7.7	9.5	10.6
$\overline{X}$	8.1	20.9	40.0	57.4	74.8	92.6
C.V.	33	28	16	13	13	11
Heart girth *(in.)*						
Single measurement						
S.D.	0.70 (0.38)	0.55 (0.63)	0.63 (0.67)	0.76	0.80	0.85
$\overline{X}$	0.82 (0.81)	1.98 (1.95)	3.49 (3.55)	4.71	6.11	7.23
C.V.	85 (47)	28 (32)	18 (19)	16	13	12
2-day average						
S.D.	0.53 (0.37)	0.50 (0.52)	0.56 (0.56)	0.66	0.73	0.75
$\overline{X}$	0.75 (0.75)	2.02 (2.01)	3.54 (3.57)	4.81	6.13	7.35
C.V.	71 (49)	25 (26)	16 (16)	14	12	10
3-day average						
S.D.	0.43	0.52	0.57	0.64	0.74	0.75
$\overline{X}$	0.78	2.02	3.57	4.79	6.13	7.33
C.V.	55	26	16	13	12	10
Girth of paunch *(in.)*						
Single measurement						
S.D.	1.03 (1.05)	1.71 (1.44)	1.37 (1.29)	1.64	1.75	1.87
$\overline{X}$	1.87 (2.04)	6.15 (6.17)	10.71 (10.72)	13.37	15.51	17.94
C.V.	55 (51)	28 (23)	13 (12)	12	11	10

C.V.	55	(51)	28	(23)	13	(12)	12		11		10	
2-day average												
S.D.	0.90	(0.95)	1.57	(1.44)	1.31	(1.18)	1.65		1.78		1.77	
$\overline{X}$	1.92	(2.01)	6.28	(6.29)	10.82	(10.83)	13.41		15.71		18.12	
C.V.	47	(47)	25	(23)	12	(11)	12		11		10	
3-day average												
S.D.	0.98		1.61		1.23		1.62		1.88		1.77	
$\overline{X}$	1.95		6.28		10.85		13.33		15.73		17.98	
C.V.	50		26		11		12		12		10	
Height at withers *(in.)*												
Single measurement												
S.D.[a]	0.35	(0.32)	0.43	(0.46)	0.48	(0.45)	0.50		0.51		0.55	
$\overline{X}$	0.98	(0.88)	1.60	(1.56)	2.34	(2.25)	2.98		3.69		4.54	
C.V.	36	(36)	27	(29)	21	(20)	17		14		12	
2-day average												
S.D.	0.27	(0.28)	0.39	(0.40)	0.44	(0.44)	0.48		0.51		0.50	
$\overline{X}$	0.93	(0.85)	1.55	(1.54)	2.28	(2.24)	2.97		3.69		4.46	
C.V.	29	(33)	25	(26)	19	(20)	16		14		11	
3-day average												
S.D.	0.26		0.38		0.41		0.45		0.47		0.46	
$\overline{X}$	0.89		1.53		2.24		2.94		3.67		4.44	
C.V.	29		25		18		15		13		10	
Length from withers to pin bones *(in.)*												
Single measurement												
S.D.	0.78	(0.80)	0.84	(0.74)	0.77	(0.92)	1.00		1.20		0.88	
$\overline{X}$	1.13	(1.14)	1.91	(1.91)	2.73	(2.68)	3.89		4.90		6.10	
C.V.	69	(70)	44	(39)	28	(34)	26		24		14	
2-day average												
S.D.	0.61	(0.63)	0.59	(0.60)	0.58	(0.64)	0.77		0.93		0.88	
$\overline{X}$	1.12	(1.12)	1.80	(1.80)	2.60	(2.58)	3.75		4.88		5.99	
C.V.	54	(56)	33	(33)	22	(25)	21		19		15	
3-day average												
S.D.	0.56		0.54		0.55		0.68		0.78		0.80	
$\overline{X}$	1.09		1.79		2.58		3.81		4.92		6.02	
C.V.	51		30		21		18		16		13	

[a] S.D. = $\sqrt{\text{Error mean square}} = s_{\bar{x}}$.

[b] Results obtained with the modified direct approach.

live weight, 31, 17, 17, and 22, respectively; for heart girth, 37, 23, 32, and 42; for girth of paunch, 40, 24, 40, and 50; for height at withers, 26, 28, 24, and 21, and for length of withers to pin bones, 38, 42, 42, and 42. It appears from these data that the coefficients of variation are largely independent of the age of the calf and are dependent primarily upon the length of the experimental period.

The coefficients of variation for growth in live weight over the 12-week period agree with the data of the Iowa Experiment Station *(10)*. Considerably higher values have been observed, however, at the Storrs Experiment Station *(9)* and at others *(11)*. Therefore, it is suggested that in calculating the number of replications needed to attain a desired experimental sensitivity, as outlined by Cochran and Cox *(7)*, 5 to 10% should be added to these values.

SUMMARY

To study the relative value of taking single-, two-, and three-day measurements in growth studies, three successive daily measurements of five growth criteria were taken on 48 calves at biweekly intervals over a period of 13 weeks, beginning on the sixth, seventh, and eighth, and ending on the 90th, 91st, and 92nd days of age. The calves were raised on a limited whole milk–calf starter system, and were grouped into eight groups of six animals each. Each group was homogeneous with respect to breed (Guernsey or Holstein), sex, and year (1951 or 1952) on experiment.

It was found that the components of variance approach for studying the value of different numbers of measurements is subject to question, because it is not safe to assume that measurements taken on successive days are independent. Accordingly, conclusions were drawn from the errors estimated by using only the first-day measurements, the averages of the first- and second-day measurements, or the averages of the measurements for three days. The Pitman-Morgan test for correlated mean squares was applied in making *t*-tests of the comparisons among single-, two-, and three-day growth measurements. Error estimates were obtained for growths over the periods one to three weeks of age, one to five weeks, ... one to 13 weeks of age.

A single-day measurement was found sufficient to determine growth in live weight, heart girth, and girth of paunch. Three-day measurements were found to be significantly better than one- or two-day measurements for length from withers to pin bones. Inconclusive results were obtained for height at withers. For each of the criteria, the size of coefficients of variation of the growth criteria decreased markedly as the length of the growth period was increased up to six weeks. Further increases in the length of the growth period yielded small additional reductions of the coefficients of variation. The sizes of the coefficients of variation appeared not to be dependent upon age of the calf, but primarily upon the length of the experimental period.

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APPENDIX TABLE 1

Effect of age on the standard deviation of a single measurement, means of the measurements, and coefficients of variation

Age		Criterion				
		Live weight	Heart girth	Girth of paunch	Height at withers	Length from withers to pin bones
(days)						
6-7-8	S.D.	1.2	0.39	0.60	0.26	0.57
	$\bar{X}$	82.2	28.52	27.92	29.03	22.32
	C.V.	1.46	1.37	2.15	0.90	2.55
20-21-22	S.D.	0.9	0.34	0.58	0.23	0.37
	$\bar{X}$	90.4	29.29	29.87	29.92	23.41
	C.V.	1.00	1.16	1.94	0.77	1.58
34-35-36	S.D.	1.2	0.32	0.62	0.19	0.39
	$\bar{X}$	103.1	30.53	34.21	30.56	24.11
	C.V.	1.16	1.05	1.81	0.62	1.62
48-49-50	S.D.	1.0	0.34	0.80	0.20	0.52
	$\bar{X}$	122.2	32.08	38.77	31.28	24.90
	C.V.	0.82	1.06	2.06	0.64	2.09
62-63-64	S.D.	1.2	0.44	0.96	0.27	0.55
	$\bar{X}$	139.5	33.31	41.25	31.97	26.12
	C.V.	0.86	1.32	2.33	0.84	2.11
76-77-78	S.D.	1.1	0.43	0.74	0.26	0.66
	$\bar{X}$	157.1	34.64	43.65	32.70	27.24
	C.V.	0.70	1.24	1.70	0.80	2.42
90-91-92	S.D.	1.5	0.47	0.81	0.24	0.65
	$\bar{X}$	174.8	35.85	45.90	33.47	28.34
	C.V.	0.86	1.31	1.76	0.72	2.29

APPENDIX TABLE 2

Effect of age on the standard deviation of a single measurement, means of the measurements, and coefficients of variation, with growth trends removed from the standard deviations

Age		Criterion				
		Live weight	Heart girth	Girth of paunch	Height at withers	Length from withers to pin bones
(days)						
6-7-8	S.D.	0.8	0.44	0.52	0.30	0.43
	$\bar{X}$	82.2	28.52	27.92	29.03	22.32
	C.V.	0.97	1.54	1.86	1.03	1.93
20-21-22	S.D.	0.9	0.27	0.47	0.24	0.37
	$\bar{X}$	90.4	29.29	29.87	29.92	23.41
	C.V.	1.00	0.92	1.57	0.80	1.58
34-35-36	S.D.	0.9	0.30	0.57	0.18	0.41
	$\bar{X}$	103.1	30.53	34.21	30.56	24.11
	C.V.	0.87	0.98	1.67	0.59	1.70
48-49-50	S.D.	0.8	0.30	0.73	0.18	0.46
	$\bar{X}$	122.2	32.08	38.77	31.28	24.90
	C.V.	0.65	0.94	1.88	0.58	1.85
62-63-64	S.D.	1.2	0.40	0.98	0.27	0.63
	$\bar{X}$	139.5	33.31	41.25	31.97	26.12
	C.V.	0.86	1.20	2.38	0.84	2.41
76-77-78	S.D.	1.0	0.37	0.61	0.26	0.58
	$\bar{X}$	157.1	34.64	43.65	32.70	27.24
	C.V.	0.64	1.07	1.40	0.80	2.13
90-91-92	S.D.	1.3	0.36	0.85	0.22	0.58
	$\bar{X}$	174.8	35.85	45.90	33.47	28.34
	C.V.	0.74	1.00	1.85	0.66	2.05

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LABORATORY STUDIES OF BLENDING LIQUIDS BY AGITATION WITH AIR

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The efficiency of agitating liquids with air was studied in glass tanks by determining with motion pictures, flow patterns, bubble size and bubble and liquid movements, and by observing time required for complete color change after adding acid to indicator solutions in the tank. Air agitation was more efficient in vertical than in horizontal tanks and it decreased at low liquid depths in both tanks. In the horizontal tank an air supply line with 12 holes uniformly spaced was most efficient for dispersing plastic particles in solutions of high viscosity, but was less efficient for those of low viscosities. For the vertical tank an air supply line with 6 holes uniformly spaced across the diameter was most satisfactory. Rapid blending occurred when a single air supply hole was placed at one-third the radius from the side of the tank. *Editor.*

Air mixing is used in the dairy industry *(2)* and certain other industries *(6, 7, 8, 15)*, but little has been published on the most satisfactory methods of application. The laboratory studies reported herein were undertaken as a basis for investigating commercial equipment.

For agitation of liquids, air is delivered through orifices in an air-supply pipe near the lowest point in a tank. At each orifice, air emerges to form a bubble, which grows until its buoyancy, assisted by the liquid movement induced by former bubbles, is great enough to detach it. As the bubbles rise, they move liquid with them. The stream of bubbles creates a column of air-liquid mixture of lower density than that of the remainder of the tank contents. Thus, the column tends to rise and be displaced by the surrounding denser fluid. The air is released at the surface, except in the case of very small bubbles, and the liquid elevated by the rising column flows toward regions of lower surface elevation. This circulation, generally turbulent, blends the tank contents.

REVIEW

Information regarding the formation and movement of bubbles in relatively still liquids is not generally applicable to this study, but some observations are pertinent. The literature contains contradictory results regarding the effect of viscosity, surface tension, and bubble size on velocity of bubbles. Much of the confusion is not of concern here, because the results were obtained in the region of laminar flow which occurs with bubbles smaller than those significant in mixing *(3, 5, 9, 11, 13, 18, 19)*. However, conflicting observations have also been reported on the behavior of large bubbles. Miyagi *(11)* studied the rise of individual bubbles formed slowly at an orifice that opened upward. Bubbles in water tended to attain a critical size (0.16-cm. radius) after release,

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the larger ones breaking up and the smaller ones coalescing until they reached critical size, if the vertical distance permitted. Within a rise of 3 to 4 cm., bubbles reached a constant terminal velocity, which was maximum (27.8 cm. per second) with bubbles of critical size. For bubbles smaller than critical size, there was a direct relation between velocity and radius. Above critical size, the larger the bubble, the lower the velocity, but for radii greater than 0.3 cm., the velocity was nearly constant at 23 cm. per second. The ratio of the mass of water carried to the mass displaced was proportionate to the radius squared, i.e., to the surface area. O'Brien and Gosline *(12)* reported rates of rise of bubbles in water, in three long tubes with diameters of 3, 5.7, and 15 cm. For small bubbles, air was introduced by small glass nozzles; for intermediate bubbles, by brass nozzles, and for large bubbles, by turning over an inverted cup which contained the desired volume of air.

The relation between bubble size and velocity for the small tubes (of 3- and 5.7-cm. diameter) was somewhat similar to that determined by Miyagi, but for the large tube (15-cm. diameter) the velocity increased continuously with bubble size. No bubble breakup was reported, even for bubbles with an equivalent radius of 2.4 cm.

Under the conditions of air mixing, bubble size is not readily controllable. Although at low rates the size of bubbles is a function of the size of the orifice through which the air is introduced, at higher rates large bubbles are produced regardless of orifice size *(10)*.

Air sparging produces a mild agitation suitable for blending miscible liquids or maintaining suspensions of solids of low settling velocities *(1)*. Literature on agitation by air sparging is scant in comparison with that dealing with other methods *(14, 16, 17)*.

Stanclift *(20)* conducted laboratory experiments in a vertical cylindrical glass tank, to investigate the effectiveness of sparging with air, in keeping solids of low settling velocities in suspension. Variables studied included sparger design, air rate, liquid depth, liquid viscosity, sold density, and particle size. The best suspension was obtained with an arc-shaped sparger concentric with the tank cross section, occupying not more than two quadrants.

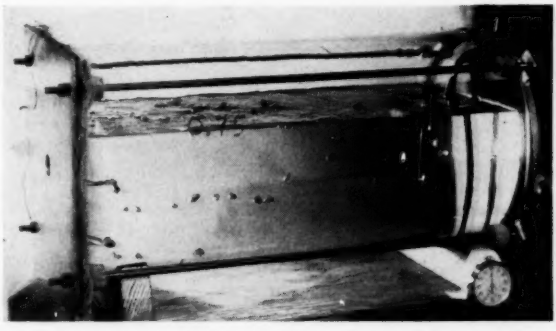

FIG. 1. Long horizontal cylindrical tank used for longitudinal blending experiments.

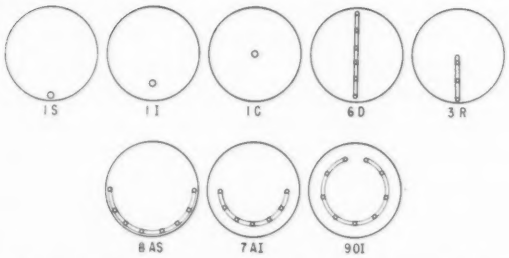

FIG. 2. Plan of supply pipes used in vertical cylindrical tank: 1S — 0.25-in. tube, at side, with open end 0.5 in. from bottom; 1I — same as 1S, but set at intermediate position (one-third radius from side); 1C — same as 1S, but set at center; 6D — straight tube on tank diameter with six holes, 0.055-in., in underside; 3R — similar to 6D, but set on radius, with three holes, 0.055-in.; 8AS — horizontal semicircular arc-shaped tube, near side, with eight uniformly spaced holes, 0.043-in., in underside; 7AI — similar to 8AS, but set at intermediate position (one-third radius from side), with seven holes, 0.043-in.; 9OI — circular tube, 9-in. diameter, with nine holes, 0.043-in.

and located two thirds of the tank radius from the center. Smaller holes appeared to be more effective than larger ones, and the best location for the holes was in the underside of the spargers. Agitation increased with depth of liquid over the range studied. Viscosity and density of the liquid, and density, size, and shape of the solid particles influenced dispersion and suspension.

Stanclift's observations covered two steps: getting the particles up from the bottom, and maintaining the suspension. His experimental procedure principally indicated the conditions most effective for the first step. The same conditions might not necessarily be the most favorable for maintaining a suspension or for dispersing material that separates to the top rather than to the bottom.

Kaufmann *(7)* recommended the quantities of air per unit of tank cross section area that are needed to give various degrees of agitation, and recognized that mixing effectiveness increased with liquid depth. The theoretical relationship developed by Glinkov *(4)* indicated that the intermixing of fluids is a function of a dimensionless criterion determined by air rate per unit of tank cross section, liquid depth, liquid viscosity, and density difference between the liquid and gas.

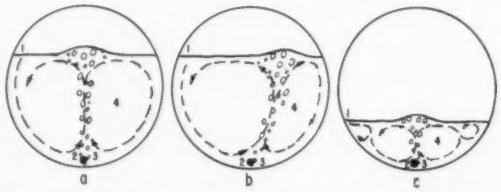

FIG. 3. Flow patterns observed in the cross section of the larger short horizontal cylindrical tank. Air inlet located 1 in. from bottom on center line.

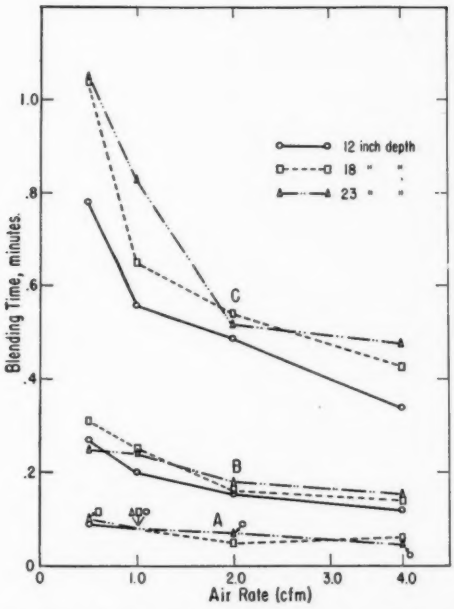

FIG. 4. Relation between air rate and successive stages of blending at three liquid depths in the larger short horizontal cylindrical tank.

In the dairy industry, air agitation is most commonly applied in horizontal cylindrical milk storage tanks *(21)*. Air is introduced through supply pipes with holes drilled in the underside, mounted just above the tank bottom. Air agitation is used to a limited extent in rectangular or vertical cylindrical milk tanks, and with ice cream mix and certain other milk products.

For agitation in film-developing tanks, Ives and Kunz *(6)* showed that with a manifold at the bottom of the tank there was a pronounced column of liquid rising in one region and descending in another. This resulted in a relatively stagnant region between the columns, which was unsatisfactory for uniform film-developing. For their purpose, they found it necessary to provide a manifold system which would introduce air adjacent to the film at a number of points throughout the tank, not simply at the bottom.

Tests on agitation in very large oil tanks for bringing cool fluid up from below the surface to extinguish surface fires were described by Risinger *(15)*. For this purpose, where intimate mixing of the contents was not required, a single central pipe introducing air at the bottom was found very effective.

EQUIPMENT

Experiments were conducted with both horizontal and vertical cylindrical tanks. Variables investigated were design of distribution manifold, air-flow

rate, depth of liquid, and viscosity of liquid. The following tanks and air-supply pipes were studied:

Larger Short Horizontal Cylindrical Tank — 36-in. diameter, 22-in. length, with glass front. A 1¼-in. air supply pipe was mounted along the bottom center line with clearance of 1 in. Four installations were tried, each with four holes uniformly spaced 5.5 in. apart. Hole sizes for three of these were 0.063, 0.094, and 0.188 in., respectively, with the holes in the underside. For the fourth, 0.063-in. holes were used in the upper side.

Smaller Short Horizontal Cylindrical Tank — 16-in. diameter, 6-in. length, with glass front and back. Air was supplied through a single hole, 0.078-in., drilled in the underside of a 0.25-in. tube mounted along the bottom center line with a 0.25-in. clearance.

Long Horizontal Cylindrical Tank — Pyrex glass jar, 11-in. diameter and 24-in. length, with a Plexiglas plate clamped to the open end (Figure 1). Air was supplied through ¼-in. pipe mounted along the bottom center line, with clearance of 0.25 in. Pipes were designated by the number and location of holes drilled in the underside, as follows:

12U — 12 holes, 0.041-in. diameter, uniformly spaced on 2-in. centers.
2E — 2 holes, one of 0.093-in. diameter, at inlet end, and one of 0.101-in. diameter, at the rear (end opposite the inlet).
2T — 2 holes, 0.093-in. diameter, one-third of tank length from the ends.
1R — 1 hole, 0.128-in. diameter, at the rear.
1T — 1 hole, 0.128-in. diameter, one-third of tank length from the rear.
1C — 1 hole, 0.128-in. diameter, at center.

Vertical Cylindrical Tank — Pyrex glass jar, 11-in. diameter and 24-in. height. The air supply pipes in this tank are illustrated (Figure 2).

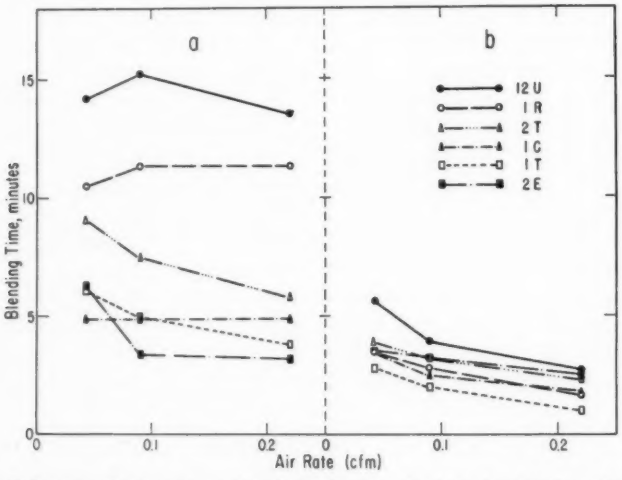

FIG. 5. Influence of air rate on blending time in the long horizontal cylindrical tank, with several air supply pipes at depth-to-diameter ratios of *(a)* 0.25:1 and *(b)* 0.75:1.

METHODS

Motion pictures were made to record bubble and liquid motion in the two short horizontal cylindrical tanks.

Blending effectiveness was measured by the time required to produce uniform color (estimated visually) after adding acid to an indicator solution. Brom cresol purple was used as the indicator, because it gave a distinct color change within a satisfactory pH range (purple at pH 6.8, yellow at 5.2). The pH of the liquid was adjusted to approximately 6.8, then sufficient acid was added to shift the pH to approximately 5.6. Care was taken that the amount of acid added would not quite produce complete color change upon mixing; otherwise, the color could become uniformly yellow before complete mixing. Most of the blending times reported are averages of duplicate determinations. If the first two results did not agree within 5% of their mean, additional measurements were made. Except for occasional values obviously in error, all values were included in calculating the average. Some results are based on as many as 11 determinations. Blending times were generally more reproducible at high than at low air-flow rates.

The air flow rate necessary to disperse polystyrene particles suspended in salt solutions of controlled densities was measured in attempts to use this suspension as a model system that would simulate the cream-in-milk system.

Air flow rates were measured with an orifice-type flowmeter. Water (at from 20 to 24° C.) was used as the liquid in all experiments, except those involving varied viscosity, for which sucrose solutions were used. Viscosity was measured with a Fisher Electroviscosimeter, and density with a Westphal balance.

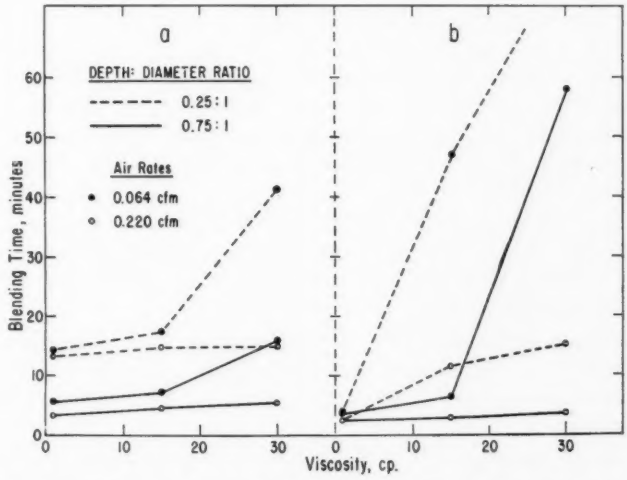

FIG. 6. Influence of viscosity on blending time in the long horizontal cylindrical tank at two liquid depths and two air rates, with supply pipes *(a)* 12U and *(b)* 2E.

RESULTS

Bubble characteristics. Bubble velocities were calculated from measurements taken from the projected motion pictures, and mean sizes were computed from air rate and bubble frequency. In the smaller short horizontal tank, bubble volume was roughly proportionate to air rate over the range studied (0.05 to 0.15 c.f.m.). Bubble frequency was thus relatively constant. Mean bubble volumes were 2.9 ml. at 0.05 cubic feet per minute, 10.3 ml. at 0.098 c.f.m., and 13.3 ml. at 0.15 c.f.m.

In the smaller short horizontal tank, the bubbles attained uniform rates of rise shortly after detachment from the pipes. Velocities were slightly greater at the higher air rates (increasing from 2.1 to 2.7 feet per second). In the larger short horizontal tank, the air rates per orifice were much greater (0.125 to 1.0 c.f.m.), but the bubble velocities were only slightly greater (2.1 *vs.* 3.1 f.p.s.).

In both tanks, some of the larger bubbles broke up during ascent, especially at the higher air rates. At higher air rates in the larger tank, extensive splashing occurred as the air escaped from the liquid surface.

Transverse blending in horizontal tanks. Flow patterns observed in the cross section of the larger short horizontal tank are shown (Figure 3). When the tank was more than half–full there were two major swirls, usually symmetrical [Figure 3 (a)]. Occasionally, the rising bubble stream swerved slowly and erratically to one side or the other [Figure 3 (b)]. With liquid depths of one-third of tank diameter or less, secondary eddies developed at the sides [Figure 3 (c)].

The successive stages of blending in the larger short horizontal tank were timed at the locations indicated by numbers (Figure 3). Timing was started when the added reagent reached the tank surface at Point 1. From there, the reagent was carried down along the tank circumference as it mixed with the swirling tank contents, and the time to reach Region 2 was recorded. These portions of the fluid, containing excess of reagent, were carried up through the center of the tank, dividing near the surface and flowing to each side. The time required to reach Region 3 on the right-hand swirl was noted. Color differences persisted longest in Region 4. Blending was judged complete when the entire contents became uniform in color.

The relation between time for blending and air rate is shown for three different depths (Figure 4). Curves A and B show the times required for added fluid, partially blended, to reach Regions 2 and 3, respectively. Times for complete blending are given by Curves C.

There were no significant differences in blending times with holes of 0.063, 0.094, and 0.188 in., respectively, in the underside, but holes of 0.063 in. in the upper side gave about 25% slower blending than did holes of the same size in the underside.

Longitudinal blending in the long horizontal tank. Flow patterns in the longitudinal section of the long horizontal cylindrical tank differed markedly from those in the cross section and were dependent on the hole arrangements

in the supply pipes. With the 12U pipe, no longitudinal flow streams were observed; blending resulted from turbulent diffusion across regions between holes. The 2E pipe produced two swirls, formed by streams flowing along the top, down the center, and along the bottom to the ends. The 2T pipe gave four swirls, with streams rising above the holes and dividing to go down at the ends and center. The two end swirls were larger than the center swirls. One swirl was produced with 1R, with flow up above the hole, along the top to the front, down, and along the bottom to the rear. The pattern produced with 1C was similar to that for 2E, with the flow direction reversed. The 1T pipe gave two swirls, similar to those with 1C but unequal in size. In most cases, the longitudinal swirls were less vigorous than the cross-sectional swirls.

The influence of air rate on blending time with different air supply pipes at low and high liquid depths is illustrated (Figure 5). Liquid depth had a marked influence on mixing effectiveness with 12U, but little influence with 2E. The influence of viscosity on blending time is illustrated (Figure 6). High viscosity caused a greater increase in blending time at the lower air rate than at the higher rate, especially with the 2E supply pipe.

The experiments with polystyrene particles suspended in salt solution gave some insight into the mixing characteristics of different supply pipes used to disperse floating particles, but the method did not provide a reliable quantitative measure of mixing effectiveness. Supply pipes 12U and 2T effected complete dispersion at the lowest air-rates, 1R was the least effective and 2E and 1C were intermediate. The plastic particles tended to cluster over regions where the liquid descended—especially at the relatively still zones at the surface over the junction of two descending streams (e.g., with 2E), or where a stream descended at a tank end (e.g., with 1R or 1C). Particles in these clusters were the last to be dispersed as air rate was increasd.

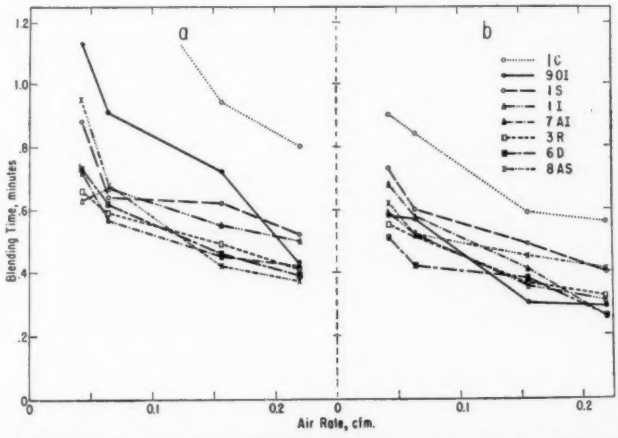

FIG. 7. Influence of air rate on blending time in the vertical cylindrical tank, with several supply pipes at depth-to-diameter ratios of *(a)* 0.5:1 and *(b)* 2:1.

Blending in the vertical cylindrical tank. The air supply pipe determined the flow pattern in the vertical cylindrical tank. Pipes 1S and 1I produced a flow that was upward from the region of the orifice and downward through a crescent-shaped column in the remainder of the tank. A central rising column was produced by 1C, with flow down around the sides. With both 8AS and 7AI, a crescent-shaped rising column resulted, and downward flow occurred only in a cylindrical region opposite the supply pipe. The flow pattern from 90I was not so distinct, but appeared to be up around the sides and down the center. Pipe 6D produced two symmetrical swirls up the diameter and down the lateral sectors. Flow with 3R was similar to 1I, but with a larger rising column.

Blending times for the vertical cylindrical tank are presented (Figures 7, 8, and 9). With all supply pipes, blending times decreased as air-rate was increased. Supply pipe 1C required the longest mixing time (Figure 7). With 90I, and to a lesser extent with 6D, blending time for water decreased with increase in liquid depth; whereas, with the other supply pipes, intermediate depths favored shortest blending times (Figure 8). Viscosity had a marked influence on blending time at low but not at high liquid depths (Figure 9).

In experiments on the dispersion of floating plastic particles, the differences between the actions with the various air supply pipes were not so pro-

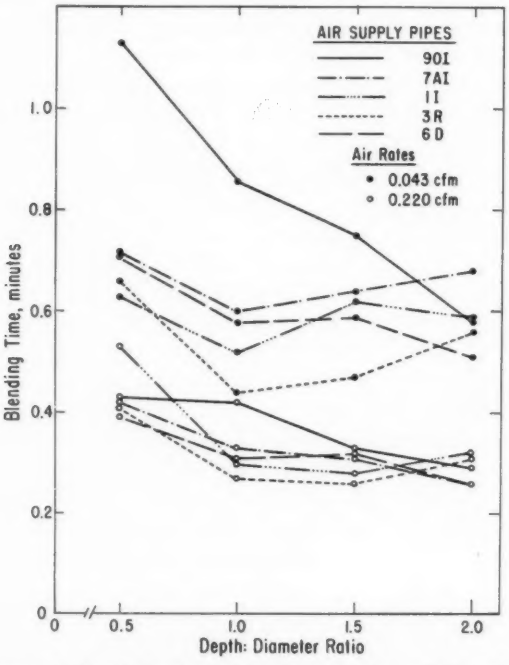

FIG. 8. Influence of liquid depth on blending time in vertical cylindrical tank, with several air supply pipes and two air rates.

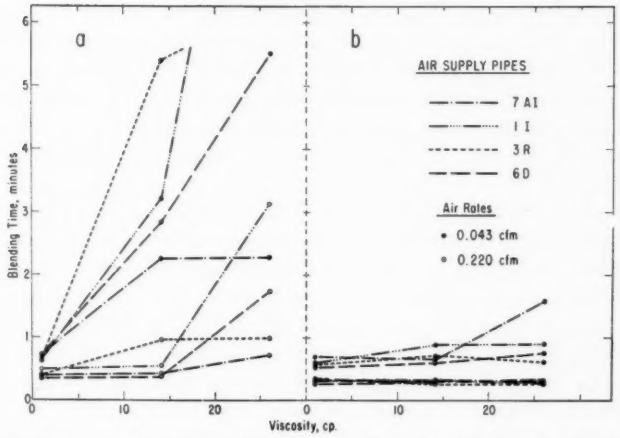

FIG. 9. Influence of viscosity on blending time in vertical cylindrical tank, with several supply pipes at two air rates and at depth-to-diameter ratios of *(a)* 0.5:1 and *(b)* 2:1.

nounced or so reproducible in the vertical tank as those observed in the horizontal tank. Clusters of plastic particles tended to form over the descending liquid column more with supply pipes 1I, 7AI, and 3R than with the others. Consequently, the former were least effective. Pipe 6D dispersed the particles at the lowest air-rate.

DISCUSSION

The bubble sizes observed were much larger than the critical sizes reported by Miyagi *(11)*. This may be attributed to the fact that with an orifice in the underside of a pipe, the buoyant force at first tends to hold a bubble against the pipe rather than to detach it. A longer-wetted perimeter is formed, which requires a greater detaching force. As the bubble grows, it becomes unsymmetrical, rises above the orifice on one side, and the buoyant force detaches it. In addition, larger bubbles are formed at higher air rates *(10)*.

The bubble velocities reported herein are much higher than those reported in the literature, but the systems are not directly comparable, because of the appreciable velocities of the liquid streams in the agitated tanks. Unfortunately, the motion pictures were not satisfactory for estimating the liquid velocities by examining the movement of nonbuoyant particles suspended in the water.

Blending in the horizontal cylindrical tanks was studied in two steps. Transverse blending occurred quite rapidly in regions near orifices. In comparison, longitudinal blending was slow. Accordingly, more attention was given to this step.

There was little tendency for end-to-end movement with the 12U supply pipe; consequently, it was slowest for longitudinal blending. The 1T, 1C, and 2E pipes produced two longitudinal swirls and, with a low depth of liquid, were more effective than the others.

In the horizontal tanks, one of the most important factors in blending effectiveness was liquid depth. Blending times were lengthened appreciably with shallow depths. In comparison, air-flow rate over the range studied was less important, especially at shallow depths. At greater depths, blending time was shortened as air-flow rate was increased at first, but additional increases gave little benefit. At shallow depths, increasing air-flow rate generally did not shorten blending times.

The relative performance of the 12U and 2E supply pipes was markedly influenced by viscosity. Although the 2E pipe was more effective in blending solutions of low viscosity, the 12U pipe was generally more effective at high viscosity. Increasing the air rate caused a marked reduction in blending times in solutions of high viscosity, especially with the 2E supply pipe. The system being agitated had a marked influence on the effectiveness of the different supply pipes in the horizontal tanks. For example, 12U took longest to blend the acid-in-water system, but was generally more satisfactory than the other pipes for blending the viscous solution and for dispersing floating plastic particles. Thus, choice of supply pipe would be influenced both by the system being blended and such conditions as liquid depth and air supply.

In general, air agitation was much more effective in the vertical than in the horizontal tank. When compared at similar air rates or fractions of fill, blending times in the vertical tank were only about one-fifth those required in the horizontal tank. Increasing air rate, over the range studied, was more effective in reducing blending time in the vertical tank than in the horizontal tank.

Low liquid depths slowed blending in the vertical as in the horizontal tanks. At depth-to-diameter ratios of one and above, the influence of depth on blending time could not be generalized, but varied with supply pipe and air-rate. Viscosity had relatively little influence on blending time at high liquid depths, but a pronounced effect at low depths, especially at lower air rates.

Of the supply pipes studied, 6D, 3R, and 7AI generally gave the most rapid blending over a wide range of conditions. Since 6D was more effective than 3R and 7AI for dispersing floating particles, it would appear to be the most satisfactory arrangement for varied conditions. The results indicate that if a single orifice is desired, locating it about one-third of the radius from the side should prove most satisfactory.

SUMMARY

Laboratory experiments with transparent liquids in glass tanks were conducted to study the formation and movement of bubbles and the factors influencing effectiveness of agitation with air. Motion pictures were taken in two short horizontal cylindrical tanks, to determine flow patterns and allow measurements of bubble size and bubble and liquid movement. To represent blending as in standardizing or fortifying, observations were made of the time required for complete color change after addition of acid to indicator solution in the tank. To simulate incorporation of a cream layer, the minimum air-rate for dispersion of slightly buoyant plastic particles was determined.

A cylindrical vessel, 11 in. in diameter and 24 in. long, placed either horizontally or vertically, was used in most tests.

Important variables were tank shape, fraction of fill, air rate, air supply pipe arrangement, and the nature of the material being agitated. Air agitation was more effective in the vertical than in the horizontal tank. In both tanks, effectiveness decreased at low liquid depths. The influence of air rate was variable: In some cases an increase markedly increased effectiveness; in others, little or no improvement was obtained. For blending solutions of low viscosity in the horizontal tank, an air supply pipe with 12 holes uniformly spaced was less satisfactory. But for blending solutions of high viscosity and dispersing the plastic-particle suspension, this air-supply arrangement was most effective. In the vertical tank, a supply pipe with six holes uniformly spaced across the diameter gave the most satisfactory results under a variety of conditions. A single air-supply hole required the longest blending times when placed at the center of the vertical tank bottom, but favored rapid blending when placed one-third of the tank radius from the side.

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MIXING BY AIR AGITATION IN HORIZONTAL MILK TANKS

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The results of laboratory experiments were applied to larger tanks and comparisons made of air and mechanical mixing. Cheese color was blended with water to study the efficiency of air and mechanical agitation in horizontal tanks. An air supply pipe with uniformly spaced holes required longer mixing than one with a single hole at the center. Mixing times decreased with increased air rate or liquid depth. For mixing a cream layer into milk, air and mechanical agitation were equally effective, but for standardizing, mechanical agitation gave uniform composition more rapidly than air agitation. Mixing cheese color into water provided useful information for blending fluids with similar physical properties, including cream and milk mixtures. *Editor.*

Some factors that influence effectiveness of mixing by air agitation in small laboratory vessels were studied previously *(1)*. This paper reports the applicability of results of the laboratory experiments to larger tanks, and some comparisons of air and mechanical mixing. Pertinent literature was reviewed in the preceding paper *(1)*.

METHODS AND EQUIPMENT

A procedure of mixing cheese color in water was used to study the influence of a number of variables on the effectiveness of agitation. Cheese color was added to water at the front of a tank at a ratio of 1 oz. per 100 gal. water; then, differences in the color of samples siphoned simultaneously from each end at desired intervals were determined by use of a Klett-Summerson colorimeter with Filter No. 42. Flow rates from the rubber siphons were adjusted so that corrections could be made for the times of passage of liquid through the tubes. Mixing times are reported as the time in minutes required to reduce the difference between samples taken from both ends to 1/25th of the mean color increase.

Similar experiments on mixing cream added to milk were conducted to determine the applicability to dairy practice of the results obtained by mixing cheese color in water. Babcock tests were used to measure composition differences, and the mixing times are expressed in the same manner as in the color-in-water experiments.

For experiments on incorporating a cream layer into milk, the mixing time reported is the minimum time required for samples taken from both ends to be consistently within 0.1% of the final test. There was no mean composition change in these experiments.

Tanks are specified by their dimensions, e.g., a 2×6.5 tank refers to one 2 ft. wide and 6.5 ft. long. Cross sections of the tanks differed. The 2×4 and 2×6.5 tanks had U-shaped cross sections with semicircular bottoms. The

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4.7 × 8.3 tank was a commercial 1,500-gal. refrigerated milk tank with oval cross section consisting of semicircular bottom and top and a mid-section with vertical sides. At depth-to-width ratios of 0.5:1 and less, these tanks are equivalent to horizontal cylindrical tanks. Experiments were made also with horizontal cylindrical tanks of customary commercial design with convex ends.

Air used for air mixing was supplied from a rotary-lobe blower, regulated by a bleeding valve, and measured by an orifice-type flowmeter. Air rates are expressed as c.f.m. per 1,000-gal. nominal capacity of a cylindrical tank of the same length and width. The air-supply pipes used are designated by a code system, consisting of a number specifying the number of holes and a letter designating their location as follows: U—spaced uniformly on 9-in. centers; C—in tank center-bottom; E—at ends; R—at rear; T—one-third of tank length from rear. The holes in the U pipes were 3/32nds in. in diameter, and other holes were drilled of such size that their total area was approximately equal to the total area of holes in the U supply pipe used in the same tank. Supply pipes were mounted longitudinally in the tanks, approximately 1 in. from the bottom, with the holes in the underside.

For the experiments involving mechanical mixing in the 2 × 6.5 tank, a "Lightnin" agitator consisting of a ⅛ h.p., 1,725 r.p.m. motor, driving a three-bladed propeller of 2.5-in. diameter, was suspended from the top of the tank in several positions. For other experiments with mechanical mixing, commercial tanks with agitators supplied by the manufacturers were used.

Rates of heat transfer through the refrigerated section of the wall of the 4.7 × 8.3 oval tank were determined for both water and milk under different conditions. The tank had 45 sq. ft. of cooling surface, for which the refrigerant flowed through channels formed by welding corrugated sheets to the 12-gauge stainless-steel wall. Temperature changes of the water or milk in the tank, and of the chilled water that was circulated, were determined, and the over-all heat transfer coefficients computed. One prolonged cooling test was made for a heat balance as a check on the accuracy of the thermal tests.

A number of experiments were conducted on a laboratory scale to compare the possible detrimental effects on milk of air and mechanical mixing. The tests were made with 1,800 ml. of milk in a 2-liter glass beaker. For air mixing, air at 0.2 c.f.m. was introduced by a glass tube 0.5-in. from the side and extending to with 0.5-in. of the bottom. To accentuate any undesirable effects, this air rate was higher in relation to milk volume than that used in other experiments. A variable-speed mechanical stirrer with a small-blade stainless-steel propeller was adjusted to give the same mixing effectiveness as measured by the colorimetric procedure used in the laboratory experiments described previously *(1)*. In most experiments, samples were agitated for 1 hr. at 4, 10, and 16° C.

RESULTS AND DISCUSSION

Experiments on mixing cheese-color in water. Representative data, illustrating the influence of tank length, air-supply pipe, and liquid depth on the time re-

TABLE 1

Influence of tank length, air supply pipe, and liquid depth on time required for mixing cheese color in water at two air rates

(Results are averages of two trials)

Tank dimensions	Depth: Width	Air-supply pipe[a]	Mixing time	
			3.3 c.f.m. air per 1,000-gal. capacity	19.7 c.f.m. air per 1,000-gal. capacity
(ft.)			*(min.)*	
2 × 4	0.5	6U	19.5	9.5
		1T	4.5	2.8
		1C	3.5	2.0
2 × 6.5	0.5	9U	34	21
		2E	18	12
		1T	14	7.0
		1C	9.5	4.5
	0.25	1C	15	9.0
	1.0	1C	5.8	2.1

[a] 6U and 9U—six and nine holes, respectively, on 9-in. centers; 1T—one hole, one-third of tank length from rear; 1C—one hole, in center; 2E—two holes, one at each end.

quired to air-mix cheese color in water, are shown (Table 1). Comparison of the results obtained with the 2 × 4 and 2 × 6.5 tanks reveals that the longer tank required a disproportionately greater mixing time than the shorter tank. Results are consistent with the previously reported laboratory experiments, in that the supply pipe with holes uniformly spaced required the longest mixing times, but they are inconsistent in that the 1C pipe was more effective than the 1T. As in the laboratory experiments, the higher air rate gave shorter mixing times, and for the 1C supply pipe, there was an inverse relation between liquid depth and mixing time.

The "Lightnin" agitator was suspended from the top of the 2 × 6.5 tank in the several positions specified (Table 2) and determinations were made of the times required for mixing cheese-color in water. Mixing was most rapid when the mixer was mounted one-third of tank length from the rear and one-third of tank width from the side, with the shaft parallel to the long side and sloped down 60° from horizontal toward the tank front. Even the poorest position of the mixer—vertical at tank center—gave a shorter mixing time than the most favorable conditions for air mixing—the 1C supply pipe with 19.5 c.f.m. air per 1,000-gal. capacity. In these tests, the power supplied to the propeller was

TABLE 2

Time required for mixing cheese color in water, using ⅛-h.p. "Lightnin" agitator mounted in 2 × 6.5 tank at depth: width of 0.5

(Results are averages of two trials)

No.	Agitator position	Mixing time
		(min.)
1	At tank center, shaft vertical	3.7
2	One-third tank length from rear on center line, shaft sloped toward center-bottom at 30° angle from vertical	2.8
3	Same as No. 2, except one-third of tank width from side	2.2
4	Same as No. 3, except shaft sloped at 45° angle	2.8

0.035 h.p., whereas that introduced with the air was 0.0085 h.p. (Power supplied to the tank contents by the air is the product of volume rate, c.f.m., times pressure, inches of water, divided by 6,356.) Results (Table 1) indicate that little increase in mixing effectiveness would have been gained by increasing the power introduced with the air. The higher air rate (19.5 c.f.m. per 1,000-gal. capacity) was greater than that normally used in the dairy industry. No tests were run with mechanical agitators that would introduce less than 0.035 h.p.

Table 3 contains results obtained in experiments with the 1,500-gal., 4.7 × 8.3 oval tank. They are similar to those presented for the 2 × 4 and 2 × 6.5 tanks. The 1C supply pipe consistently gave shorter mixing times than the 11U supply

TABLE 3

Time required for mixing cheese-color in water in the 4.7 × 8.3 oval tank, using air under varied conditions and the mechanical agitator

Supply pipe [a]	Air-rate c.f.m. per 1,000-gal. capacity	Mixing time at depth:width			
		0.25	0.5	0.7	1.2
			(min.)		
11U	0.9		26	14	
	2.8	50	18	10	5
	4.6		12	9	
1C	0.9		6		
	2.8	10	5.5	4.5	3
	4.6		5.5		
Mechanical [b]			1.0	1.5	1.5

[a] 11U—11 holes, spaced 9 in. apart; 1C—one hole in center.
[b] End-entering agitator with ¾-h.p. motor.

pipe. With both supply pipes, there was an inverse relation between liquid depth and mixing time. Decreasing the air rate from 4.6 to 0.9 c.f.m. per 1,000-gal. capacity made relatively little difference in mixing time for the 1C supply pipe, but approximately doubled the time required for the 11U supply pipe. The mechanical agitator mixed cheese color into water more rapidly than did air under any of the conditions studied. Its ¾-h.p. motor required 360 w. for 0.23 shaft h.p. For 28 c.f.m. at 1.5 p.s.i., the air h.p. was 0.016. This could have been supplied by a ⅓-h.p. motor using 190 w.

Comparisons of air and mechanical mixing by the color-in-water procedure were made also with two 4,000-gal. commercial horizontal cylindrical tanks. Mixing time for a half-filled 7.5 × 12 tank, agitated by means of a top-entering agitator driven at 96 r.p.m. by a ½-h.p. gear-reduction motor, was 2 min. In contrast, 28 min. were required for comparable mixing by air in a half-filled 7 × 14 tank agitated by 1.7 c.f.m. air per 1,000-gal. capacity, supplied by a compressor equipped with a ¾-h.p. motor and introduced through an 18U supply pipe.

Special consideration was given to studying the rate of air mixing in horizontal cylindrical tanks, using supply pipes that distribute the air uniformly along the tank bottom. Colorimeter readings obtained in mixing tests in the

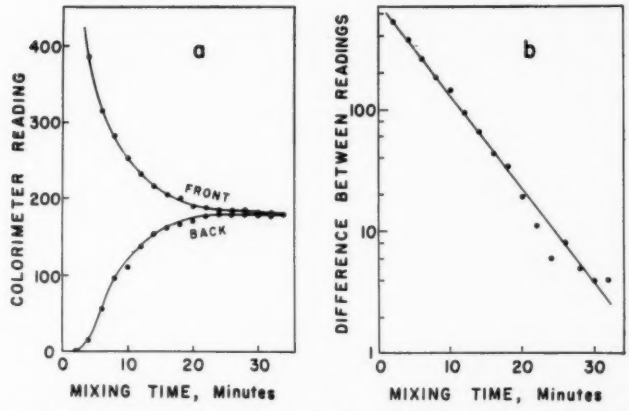

FIG. 1. *(a)* Colorimeter readings of samples taken from front and back of air-agitated horizontal cylindrical tank during mixing of cheese-color into water. *(b)* Difference between front and back readings plotted on semilogarithmic coordinates.

7×14 tank half-filled with water are plotted with arithmetic scales [Figure 1 (a)]. The differences, plotted on semilogarithmic coordinates against time [Figure 1 (b)], fell approximately on a straight line. The same relationship was observed in other tests with this type of supply pipe. This confirms the presumption that the rate of change of composition difference is proportionate to the difference. Obviously then, the time required for mixing with the supply pipe with uniformly spaced holes depends both upon the mean composition change and upon the minimum uniformity acceptable.

Comparisons were made of mixing times for tanks ranging in capacity from 10 to 7,000 gal., agitated by air introduced through supply pipes with uniformly spaced holes. Results indicated that tanks with the same ratios of length to width require roughly comparable mixing times if operated at the same air rate expressed as c.f.m. per 1,000-gal. capacity. In commercial practice, an air rate of about 2 c.f.m. per 1,000-gal. capacity is common.

Experiments on mixing cream in milk. Experiments were conducted to compare the mixing of cream into milk with the mixing of cheese color into water, using both the 2×6.5 and the 4.7×8.3 tanks. Cream was introduced into the front of both tanks, through a 1-in. sanitary pipe extending to within 1 in. of the bottom in the 2×6.5 tank, and through a "no-foam" inlet in the 4.7×8.3 tank. The results (Table 4) show that there was satisfactory agreement between the mixing times in the 2×6.5 tank, but not in the 4.7×8.3 tank. A possible explanation for this discrepancy is that the cream introduced through the no-foam inlet may have flowed along the surface of the milk, which would have favored longitudinal distribution. In any case, the discrepancy did not influence the order of rank of the mixing effectiveness of the three methods. These results indicate that blending cheese color in water can provide helpful information for comparing different methods of blending fluids with similar physical properties.

TABLE 4

Mixing times required for blending cheese color in water and cream in milk in two tanks by means of air and mechanical mixing

(Depth : width = approx. 0.5.)

Tank	Method of agitation	Mixing time	
		Cheese color in water	Cream in milk
		(min.)	
2 × 6.5	Air, 3.3 c.f.m./1,000-gal. capacity, 9U supply pipe	34	24
2 × 6.5	Air, 3.3 c.f.m./1,000-gal. capacity, 1C supply pipe	9.5	10
2 × 6.5	Mechanical—Lightnin' agitator	2.2	2.5
4.7 × 8.3	Air, 2.8 c.f.m./1,000-gal. capacity, 11U supply pipe	18	8
4.7 × 8.3	Air, 2.8 c.f.m./1,000-gal. capacity, 1C supply pipe	5.5	2.5
4.7 × 8.3	Mechanical end-entering agitator	1.0	1.5

Conditions involved in incorporating a cream layer from the surface of milk differ markedly from those provided in the blending experiments described above. Under these conditions, the cream is relatively uniformly distributed over the surface of the milk into which it is to be incorporated. Also, it may have appreciable structural viscosity, which makes mixing more difficult. Results of a number of tests made to compare the effectiveness of air and mechanical mixing, for incorporating a cream layer formed on milk while standing undisturbed in the 4.7 × 8.3 tank for 20 hr. at approximately 6° C., are shown (Table 5). With similar milk volumes, the 11U supply pipe consistently required shorter mixing times than the 1C. At lower liquid depths, both supply pipes required longer mixing times. Tests were not made with the mechanical agitator when the milk depth was insufficient to submerge the agitator. When completely submerged, the mechancial agitator gave results similar to those for air mixing, using the 11U supply pipe with 2.8 c.f.m. air per 1,000-gal. capacity.

TABLE 5

Time required for incorporating a cream layer into milk in the 4.7 × 8.3 oval tank, mechanical agitation compared with air at 2.8 c.f.m.

Agitation	Weight of milk	Depth : width	Mixing time
	(lb.)		*(min.)*
Air, 11U	2,300	0.3	12
	2,800	.3	7
	3,600	.4	7
	4,100	.5	3.5
	4,300	.5	2.5
	5,400	.6	2.5
	6,600	.6	1.5
Air, 1C	3,400	.4	20
	3,500	.4	8
	4,300	.5	7
	6,300	.6	3
	6,550	.6	6
Mechanical	4,300	.5	2.5
	6,000	.6	3.5
	6,400	0.6	2

Consideration must be given to the mixing characteristics desired when selecting a method of agitation. The blending experiments simulate conditions involved in standardizing the fat percentage of milk by addition of cream or skimmilk, or in fortifying by addition of a vitamin concentrate. For blending, mechanical agitation gave better results than air. With air, the 1C supply pipes were more satisfactory than those with holes uniformly spaced 9 in. apart.

The experiments on incorporating a cream layer are more representative of the conditions encountered in milk storage tanks, where the prime requisite is gentle agitation that will prevent a cream layer from forming, or that will incorporate an existing cream layer, but where end-to-end mixing is relatively unimportant. For incorporating a cream layer, the limited tests with commercial equipment indicated that mechanical agitation and air agitation with the U-type supply pipe were roughly comparable in effectiveness, and that the U supply pipe was more satisfactory than the 1C. The principal limitation of mechanical agitation is that it is ineffective at low milk depths that do not submerge the blades. Air agitation can be used with lower milk depths than can commercial mechanical agitators in horizontal tanks, but even air agitation is relatively ineffective at low milk depths.

TABLE 6

Effect of method of agitation, air rate, and liquid depth on heat transfer in the 4.7 × 8.3 oval tank

Agitation	Air rate	Fluid	Depth: width	Over-all heat transfer coefficient B.t.u/hr/ft²/° F.
	(c.f.m.)			
Air, 11U	0.9	Water	0.5	40
	2.8			46
	4.6			49
	0.9		0.7	41
	2.9			47
	4.6			50
	1.8		1.2	52
	2.9			54
	4.6			56
	0.9	Milk	0.7	31
	2.8			38
	4.6			43
Air, 1C	0.9	Water	0.5	42
	2.8			47
	4.6			51
	0.9		0.7	42
	2.8			48
	4.6			51
	0.9		1.2	42
	2.8			47
	4.6			50
	2.8	Milk	0.6	41
	2.8		0.7	41
Mechanical		Water	0.5	55
			0.7	58
			1.2	57
		Milk	0.6	51
			0.7	52

Heat transfer experiments. Results of heat transfer tests under different agitation conditions are presented (Table 6). Heat transfer coefficients for both the 11U and 1C supply pipes increased about 20%, with an increase in air rate from 0.9 to 4.6 c.f.m. Depth of water made little difference in the heat transfer coefficient with the 1C supply pipe, but with the 11U supply pipe a full tank gave about 20% greater thermal conductance than the partially filled tank. Mechanical agitation gave roughly 10% higher heat transfer coefficients than air at the highest rate. With mechanical agitation, the heat transfer coefficient was reduced when the agitator was not completely submerged. Conductance with milk was about 85% of that with water.

Comparison of detrimental effects of air and mechanical mixing. A comparison of air and mechanical mixing for possible detrimental effects indicated that incidence of oxidized flavor was not related to method of agitation. Distinct feed flavor was very slightly reduced by air agitation. This was fairly consistent, but the reduction was so slight that it would be of little importance in commercial operations. Rancid flavor was encountered frequently in samples agitated by air at 16° C., but not in the mechanically agitated samples, the nonagitated samples, or in any of the samples treated at 4 or 10° C.

Ascorbic acid destruction was slightly greater at 16° C. in the air agitated samples than in those agitated mechanically, but at lower temperatures there were no differences, either immediately after mixing or after storage at 4° C. for 48 hr. No consisten differences between air mixed and mechanically mixed samples were observed with respect to creaming properties or susceptibility to cream plug formation. Oxygen, as determined by the ascorbic acid oxidase method, was not increased as a result of air-mixing raw milk received by conventional methods and stored 20 hr. at 5° C. in the 1,500-gal. oval tank in the University Creamery.

SUMMARY AND CONCLUSIONS

The results reported emphasize that consideration must be given to the mixing characteristics desired, when selecting a method of agitation.

Cheese color was blended into water to study the influence of a number of variables on the effectiveness of air agitation and mechanical agitation in horizontal tanks. Results were consistent with laboratory experiments reported previously, in that an air supply pipe with uniformly spaced holes required longer mixing times than one with a single hole at the center. In general, mixing times decreased with increase in air rate or liquid depth. A long tank required a disproportionately greater mixing time than a short tank of the same width. In experiments comparing air agitation and mechanical agitation, the latter consistently achieved shorter mixing times.

Experiments to compare the mixing of cream into milk with the mixing of cheese color into water indicated that the color-in-water procedure can provide helpful information for comparing different methods of blending fluids with similar physical properties.

In experiments on incorporating a cream layer into milk, similar mixing times were required for mechanical agitation and for air agitation using a supply pipe with uniformly spaced holes. In these tests, a supply pipe with one hole in the center required appreciably longer mixing times and gave less consistent results.

The mechanical agitator supplied with the 1,500-gal. oval tank gave higher heat transfer coefficients than did air agitation at the highest air rate used.

Air mixing and mechanical mixing, when compared in laboratory-scale experiments, showed few differences in detrimental effects on milk.

REFERENCE

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VARIATIONS OF SCORES AMONG DIFFERENT CHARACTERISTICS OF HOLSTEIN COWS FOR HERD-TYPE CLASSIFICATION [1]

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This investigation compared the different points on the score-card for classifying Holsteins over a five-year period and determined the uniformity with which they were rated by different inspectors. With over-all ratings, 26.16% of the total variation was due to variations among inspectors and the percentage was greater for all other characteristics. Average correlation between over-all ratings was +.69. General appearance, rump, and body capacity were more uniformly rated than the mammary system, dairy character, and feet and legs. Consistency of ratings varied widely for the different characteristics. *Editor.*

In herd-type classification work, not all characteristics of the animals, as listed on the simplified score-card, can be judged, evaluated, and scored with the same efficiency and consistency. The object of this investigation, therefore, was to identify and compare the different characteristics according to the degree of uniformity and efficacy with which they could be rated under practical conditions.

A number of studies in the past have shed some light on the subject. Gowen *(2)* reported in 1923 that inspectors differed most in scoring animals for general appearance, size and character of udder, and size and shape of fore udder, rump, and barrel. They agreed more in scoring shape of udder and body wedge. Johnson and Lush *(5)* concluded that genuine differences apparently existed in the ideals of the official judges, but they were uncertain if these could be eliminated by more conferences among judges. Hyatt and Tyler *(4)* were of the opinion that much of the wide variation in type ratings could be attributed to the inability of the inspectors to attach equal significance to certain faults of the cow. Most agreement was found in the appraisal of characteristics other than feet, legs, and udders.

In Ayrshire cows, results of a study by Benson, Tyler, and Hyatt *(1)* showed a range of correlations of from + 0.61 to + 0.76 for over-all ratings made at the same time by different inspectors. Similarly, correlation coefficients of break-down ratings ranged from +0.10 to + 0.77. Touchberry and Tabler *(8)* noted a correlation of + 0.62 when ratings were made on the same cow at the same time by different men. Harvey, Ross, and Fourt *(3)* obtained a correlation of + 0.64 in a similar study on heifers. McGilliard and Lush *(6)*

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completed a study of a similar nature which indicated that genuine differences in ideals were quite small among judges. A correlation of + 0.73 resulted from a study of over-all ratings made by different judges on the same cow at the same time.

EXPERIMENTAL PROCEDURE

Data covering five years, from 1945 to 1950, were available for study from official type classifications of the Holstein-Friesian herd owned by the New Jersey Agricultural Experiment Station. During this time, all females of milking age were classified twice annually, classifications generally taking place in May and October at the beginning of the pasture- and winter-feeding seasons, respectively. Three official inspectors, working individually and independently, classified each animal at the same time and under identical conditions. They made a total of 1,575 appraisals of 525 adult females during the ten classifications for each of the various type components. For ease of analysis and maintenance of equal intervals between ratings, the classification ratings were coded as follows: "Excellent" rated as six, "Very Good" as five, "Good Plus" as four, "Good" as three, "Fair" as two, and "Poor" as one. This method was proven sound in a study of methods of estimating heritability of type classifications by Tabler and Touchberry *(7)*; a modification was used by McGilliard and Lush *(6)*.

During the period covered by the study, nine different official type classifiers made the type ratings. All appeared at least twice; one appeared seven times. Because of this fact, variation due to differences among animals and among inspectors is presented on a within-classification basis. Standard analytical procedures were used in the various statistical analyses. Correlation coefficients were averaged through use of Z transformation values.

RESULTS

Part I. Variance analysis. By analysis of variance, the different sources of variation were divided as follows: among classifications, among animals, and among inspectors. Results of this analysis are presented (Table 1), where the actual and percentage component values of the total variation, for all ratings and subratings, are shown. Also, for convenience' sake, the characteristics have been ranked according to the apparent increase in difficulty of uniform evaluation.

It is evident that a part of the total variation is made up of the influence of the different classifications. Much of this variation can be attributed to season and year effects, there being differences in stage of pregnancy and lactation, and cow replacements and number of cows, from season to season and year to year. These factors influenced the ratings of all inspectors and probably accounted for most of the among-classification variation. Also, there may well have been time-trends and other miscellaneous influences which helped create this variation. These factors were not isolated for this study and accounted

TABLE 1

Existing total variance of type ratings in ten classifications of the New Jersey Experimental Holstein Herd

Characteristic	Among classifications		Within classifications							
			Among animals		Among inspectors					
					Consistent		Inconsistent		Sum	
	(σ^2)	(%)	(σ^2)	(%)	(σ^2)	(%)	(σ^2)	(%)	(%)	(*Rank*)
Over-all rating	0.074	9.05	0.530	64.79	0.010	1.22	0.204	24.94	26.16	1
General appearance	.068	8.12	.517	61.69	.008	0.95	.245	29.24	30.19	2
Dairy character	.041	5.28	.385	49.55	.046	5.92	.305	39.25	45.17	8
Body capacity	.035	3.93	.547	61.39	.031	3.48	.278	31.20	34.68	4
Mammary system	.081	10.04	.402	49.81	.029	3.59	.295	36.56	40.15	5
Fore udder	.090	8.99	.495	49.45	.037	3.70	.379	37.86	41.56	6
Rear udder	.079	9.52	.393	47.35	.021	2.53	.337	40.60	43.13	7
Rump	.062	5.35	.725	62.61	.025	2.16	.346	29.88	32.04	3
Feet and legs	0.112	10.17	0.458	41.60	0.126	11.45	0.405	36.78	48.23	9

for between only 3.93 and 10.17 per cent of the total variation for the different characteristics.

It was apparent, in general, that the largest component of variance was the among-animal-within-classification variation. This indicated that valid differences existed among the type ratings of the individuals within each classification. A range of from 41.60 to 64.79% was evident for this part of the total variation.

The last source of variation was the among-inspector-within-classification variation. This value indicated the degree of difficulty experienced by well-trained official-type classifiers in giving a true evaluation of the individual animals. It, in turn, was broken down into two parts. The consistent part measured the effect of the differences in mean levels of the ratings of the individual inspectors. In each classification, the herd-wide average score of each inspector was slightly different from that of either of the other two inspectors. This effect was generally small, although it did amount to 11.45% in the case of feet and legs. In the case of general appearance, it amounted to only 0.95%.

The remaining factor was the inconsistent source of variation. This corresponded to the residual or error term in the analysis of variance. Since the inspectors rated the animals at the same time and under the same conditions, this term was a measure of the human error, or inconsistency, within the individual inspectors. It was caused by a fluctuation of scoring, in that the score of an inspector would be higher, between, or lower than the two other inspectors, and this relationship would change from animal to animal. Whether this was a within- or among-classifier fluctuation, or both, could not be investigated.

When the consistent and inconsistent variations were totaled, there resulted the best estimate of the difficulty in making this kind of subjective appraisal. These totals were ranked and presented valuable comparisons. It was apparent

that several of the subratings were more difficult to appraise than others. Over-all rating, general appearance, and rump appeared to be the easiest to score uniformly, while feet and legs, dairy character, and rear udder were the most difficult. As an example, almost twice as much success was obtained in making a valid appraisal of the over-all rating than was obtained with legs and feet.

Many breeders, when they classify, may not be interested in the variation due to different classifications. In this case, the variation among classifications may be subtracted from the total variation. Thus, the portions of the other sources will be increased proportionally. This adjustment is shown (Table 2). Such a shift brought the distribution of the variation into appropriate focus

TABLE 2

Existing variance in percentage of type ratings when limited to among cows and among inspectors

Characteristic	Among animals	Among inspectors			
		Consistent	Inconsistent	Sum	
			(%)		*(Rank)*
Over-all rating	71.24	1.34	27.42	28.76	1
General appearance	67.14	1.04	31.82	32.86	2
Dairy character	52.31	6.25	41.44	47.69	8
Body capacity	63.90	3.62	32.48	36.10	4
Mammary system	55.38	3.99	40.63	44.62	5
Fore udder	54.34	4.06	41.60	45.66	6
Rear udder	52.33	2.80	44.87	47.67	7
Rump	66.15	2.28	31.57	33.85	3
Feet and legs	46.31	12.74	40.95	53.69	9

and shed more light upon the characteristics. The rankings of various components were not altered by this adjustment.

Part II. Correlation analysis. By simple correlation procedures it was possible to obtain further evidence. While the analysis of variance presented evidence of the degree of difficulty in appraising the various type components, correlation procedure resulted in estimates of the success obtained in making the various appraisals. One estimate, then, was an approximate complement of the other.

Results of these analyses are presented (Table 3), where the ranges of all correlations are shown for each of the characteristics among *(a)* all inspectors within classifications, and *(b)* all classification means, with each based on the average of the three inspectors. In addition, there is shown the average correlation for each characteristic, which has been computed from 30 individual correlations (three comparisons between individual inspectors times ten classifications). The combining techniques used in the correlation procedures probably had a slight smoothing effect upon the final figures.

The relationships among inspectors ranged from a relatively low correlation (+0.220 for feet and legs) to a quite high correlation (+0.927 for rump). The range of the correlations among classifications was less pronounced (+0.342 for

TABLE 3

Ranges and averages of correlation coefficients of type ratings among all inspectors and among classification means

Characteristic	Range among all inspectors		Range among classification means		Average of all inspectors and/or classifications means	
	Low	High	Low	High		
			(*r*)			(*Rank*)
Over-all rating	0.424	0.810	0.604	0.755	0.694	2
General appearance	.446	.894	.552	.775	.672	4
Dairy character	.326	.735	.361	.682	.560	7
Body capacity	.352	.857	.534	.809	.683	3
Mammary system	.326	.762	.492	.685	.583	5
Fore udder	.239	.784	.415	.733	.567	6
Rear udder	.313	.733	.391	.614	.544	9
Rump	.392	.927	.556	.797	.752	1
Feet and legs	0.220	0.731	0.342	0.718	0.545	8

feet and legs to +0.809 for body capacity). The over-all average correlations constituted the best estimates of the success in making true appraisals of the characteristics.

DISCUSSION

Analysis of the over-all scores, known as classification rating on the official score-card, yielded estimates noticeably similar to other reported studies. The correlation, of 0.694, of over-all ratings made by different judges on the same cow at the same time, seems to be well-supported and falls in the midst of comparable correlations mentioned earlier.

Since the over-all rating is primarily an average of the various type components, it is logical to assume that greater consistency would be evidenced in its evaluation. Such was the case in this study. Analysis of variance showed the least human error involved of all the characteristics, and the correlation coefficient ranked second only to rump in value. The real differences among animals within classifications accounted for 64.79% of all the variation.

General appearance was also quite uniformly evaluated. Since it is rather generally accepted to be closely associated with over-all rating, the high degree of uniformity in its evaluation seems quite logical. It was second lowest in amount of error attributed to inspectors, ranking fourth in correlation between ratings.

Of possibly the least economic importance, and valued only esthetically, is rump. Its ratings are quite highly correlated; it appears to be among the easiest of the characteristics to evaluate.

A correlation of 0.683 and a fourth-place ranking in the analysis of variance showed that body capacity, certainly positively correlated with milk production, was quite uniformly rated. The consistent source of variation, the part due to the difference in average levels of the judges, accounted for only 3.48% of the total.

Mammary system and its two breakdown components fore udder and rear udder are among those difficult to evaluate. It is here that the total variation

among inspectors approaches in size the among-animal variation. These three score-card items rank fifth, sixth, and seventh in the analysis of variance, and fifth, sixth, and ninth among the correlation coefficients, respectively. Although the general levels of the inspectors' ratings were quite similar, it is here that a smaller fluctuation of ratings would result in a markedly higher agreement among the ratings. Mammary system counts for 30 points on the score-card. Its relative importance, then, is quite evident.

An even greater difficulty in rating is apparent with dairy character. Because of the more indefinable quality of this characteristic, the lower correlation among ratings is quite explainable. This characteristic counts 20 points on the score-card. Further study to describe and isolate this characteristic might well do much to increase the uniformity of its evaluation. There is also an apparent difference in leniency attached to this characteristic. An average of 5.92% of the total variation is attributable to the differences in average levels of the judges.

Ranking lowest in the analysis of variance and eighth in the correlation analysis is feet and legs. Due in part to the fact that 11.45% of all the variation is due to a difference in average rating levels, the total among-inspector variation actually amounts to more than the among-animal variation. Not only should inspectors work more toward agreement in average levels, but also study is needed in reducing the human-error element involved.

With over-all rating an average of the various characteristics, it seems safe to say that improvement can be made by emphasizing those shown to be the more difficult to evaluate. The characteristics that are the most subjective in their evaluation, namely dairy character and mammary system, show the least amount of agreement in their appraisal.

SUMMARY

A study of records available covering a five-year period was undertaken to determine the causes of the variations in scores among different characteristics of Holstein cows in herd-type classification. During this period three inspectors, simultaneously and independently, semiannually rated each animal. A total of 1,575 observations of 525 adult females was studied for each of the various characteristics.

Analysis of variance resulted in measures of the variations among classifications, among animals with classifications, and consistent and inconsistent variations among inspectors within classifications. Ranges and averages of correlations between scores of the same animal at the same time were presented for each of the type components.

With over-all rating, 26.16% of the total variation could be attributed to variation among inspectors within classifications. The percentage was greater with all of the other characteristics. Average correlation between over-all ratings was +0.694. Rump, general appearance, and body capacity are more

uniformly evaluated than mammary system (with fore and rear udder), dairy character, and feet and legs.

Should subsequent studies indicate positive relationships between type ratings and/or subratings and characteristics of direct economic importance, additional study along lines leading to greater agreement among inspectors is warranted. In this investigation, consistency in rating varied widely among the different characteristics. Although progress seems to have been made in this respect during past years, additional effort to standardize appraisal methods for those characteristics shown to be the most difficult to evaluate uniformly, such as dairy character and feet and legs, holds the greatest promise for improvement.

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REMOVAL OF OXYGEN FROM EVAPORATED MILK WITH GLUCOSE-OXIDASE[1]

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Oxygen was removed from evaporated milk with glucose-oxidase by incubating and agitating it for from 24 to 48 hr. Oxygen was decreased below 0.002-ml. per can of evaporated milk with glucose-oxidase at 40° F. for 40 hr. Levels of 0.004-ml. per can were obtained by processing the milk in carbon dioxide. These low oxygen levels did not affect the browning of milk by sterilization and storage. *Editor.*

Previous studies *(5)* have indicated that the color and flavor of evaporated milk after sterilization are significantly improved by reducing the oxygen content. Methods of reducing oxygen and of determining its traces have recently been improved. In the present study, oxygen was reduced to much lower levels than those previously obtained *(5)*. This was done by using enzyme glucose-oxidase or by keeping the evaporated milk in a gas of low oxygen content during processing.

EXPERIMENTAL PROCEDURE

Determination of oxygen. The total oxygen in a can of evaporated milk seems to be the best quantity to consider in determining the role of oxygen in the development of color and flavor during sterilization and storage. Oxygen in the free space equilibrates with that in the liquid according to Henry's law. As the latter oxygen is used in the browning reaction, the former dissolves into the milk to maintain equilibrium. Since determining oxygen in the free space yields a fair approximation of the total oxygen per can of evaporated milk, this procedure was preferred over determining total oxygen directly, which is more complicated. In a standard can (14½ oz.) at equilibrium, with 20 ml. free space, 52, 67, and 80% of the total oxygen per can is in the free space at 40, 70, and 90° F., respectively. Percentages were calculated from the solubility of oxygen in milk *(1)* and water.

For determining traces of oxygen, the method of Pepkowitz and Shirley *(3)* was adopted with slight modification. This method is based on the following stoichiometric reactions:

$$(1)\quad 4\,Mn(OH)_2 + O_2 + H_2O = 4\,Mn(OH)_3$$
$$(2)\quad 2\,Mn(OH)_3 + 2I^- + 6H^+ = 2\,Mn^{++} + I_2 + 6\,H_2O$$
$$O_2 = 2I_2$$

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The amount of I_2 extracted with toluene is determined spectrophotometrically. Difference in iodine equivalent to 0.0001-ml. of oxygen can be measured spectrophotometrically. The accuracy of the method, limited by other phases in the procedure, was of magnitude 0.001-ml. of oxygen.

The method was modified by evacuating the acid in the dissolver before the preparation of manganous sulfate. This allowed omission of a trap required to remove entrapped air with the first few milliliters of solution. A plug of Pyrex glass wool at the bottom of the dissolver prevented particles of the powdered manganese metal from entering the reaction bulb.

Two sources of error caused high values: Iodine forming from potassium iodide in air during extraction in a separatory funnel, and the dissolving in toluene of Apiezone N grease, resulted in increased absorption. Small particles of grease, loosened from stopcocks and joints, floated on the liquid; this could not be avoided. Iodine formation by air was eliminated by admitting 4.0 ml. of toluene into the evacuated bulb and extracting iodine in vacuum. Air was admitted after the two layers had formed, and the top layer was recovered in a separatory funnel. Grease was eliminated by distilling the toluene extract in vacuum with the receiver cooled by dry ice in alcohol. This procedure did not yield 100% recovery for three reasons: *(1)* the extraction was not quantitative, *(2)* part of the iodine was lost during the distillation, and *(3)* some iodine was adsorbed by the grease remaining in the distillation flask. However, good duplicate values were obtained. The blank value, 0.001-ml. of oxygen, was subtracted.

The method was tested by admitting oxygen as air of low pressure, from a gas burette into the apparatus by mercury displacement. It was found that considerable time was required for such small quantities of gas to equilibrate from the admission line over the reaction bulb. Therefore, a method was tried in which the known amount of air was followed by 10 ml. of nitrogen, to push it into the reaction bulb. The values were corrected for the oxygen content of the nitrogen as determined by the Pepkowitz-Shirley method. Recoveries from admitted 0.010- and 0.020-ml. of oxygen at 32° F. and a pressure of 76 cm. of mercury were approximately 60%. About 80% iodine was recovered by distilling solutions of iodine in toluene made up in similar quantity and concentration. This left 80-60 = 20% recœovery lost by incomplete extraction and adsorption of iodine by the grease.

For determination of oxygen in cans of evaporated milk, the admission line for the gas sample was connected with a device sealed gas-tight on the can and designed to punch the lid and admit the gas from the free space into the apparatus. The sample represented about 98% of the gas in the free space, because the gas from 20 ml. of free space was admitted into a one-liter reaction bulb. No transfer of oxygen from milk into free space was assumed during the short period of taking the sample (results indicate a slow rate of transfer). A recovery of 60% was adopted, to calculate the quantity of oxygen in the free space of the can from the value obtained via determination as above. The total oxygen per can was calculated considering volume of the free space and temperature.

Removal of oxygen by glucose-oxidase. Evaporated milk (14½-oz. cans) in a commercial plant was taken off the conveyor before sterilization and immediately placed in ice-water. The experiments were started within a few hours. Aqueous solutions of enzyme and glucose were added to the milk through a hole punched in the lid and the cans were immediately sealed and incubated. Quantities added were 0.01% deoxygenase[3] and 0.3% glucose (percentages of the evaporated milk) in 2 and 3 ml. solution, respectively. The reaction is:

$$(1)\ \text{glucose} + O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \text{gluconic acid} + H_2O_2$$

$$(2)\ H_2O_2 \xrightarrow{\text{catalase}} H_2O + \tfrac{1}{2}\,O_2$$

The net reaction is:

$$\text{glucose} + \tfrac{1}{2}\,O_2 \xrightarrow{\text{enzyme}} \text{gluconic acid.}$$

Oxygen is removed by the reaction in the presence of glucose. Preliminary experiments indicated that removing all the oxygen from evaporated milk packed in air would require long incubation periods at optimum temperature (from 70 to 100° F.). Using 1% glucose and 0.1% enzyme, incubation at 90° F. for 2½ and 10 hr. reduced the oxygen per can to 0.238-ml. and less than 0.001-ml., respectively. These incubation periods were too long to be applicable to evaporated milk, because quality and heat stability are reduced by growth of organisms.

Experiments were continued with nitrogen-packed milk, varying the time and temperature of incubation. For incubation at 40° F., packing in air was also included. The milk was packed in nitrogen by placing the cans, opened by punching holes in the lids, in a vacuum desiccator, evacuating to a pressure of 3 cm. of mercury, and then filling with nitrogen four times in succession. The solutions were then added by pipette, and the cans were immediately sealed through a small hole in the lid of the desiccator while nitrogen was continually flowing out. Incubation followed in a Fort Wayne batch sterilizer completely filled with water and held at incubation temperature (90, 70, or 40° F.). Cans were agitated during incubation by operating the reel continually at 7 r.p.m.

Oxygen was determined[4] immediately after incubation and again after holding for one day at 40° F., if incubation was at a higher temperature. The rest of the cans were sterilized at 243° F., as described previously *(4)*. Determination of oxygen was repeated after sterilization and holding of the cans for one day at 40° F., to obtain equilibrium between oxygen in evaporated milk and free space at 40° F.

Keeping the condensed milk in a gas of low oxygen content during processing. The idea behind this method is that milk can be freed from oxygen by con-

[3] "Dee-O" a mixture of glucose-oxidase and catalase, was obtained from Takamine Laboratory, Inc., Clifton, N. J.

[4] In this case, oxygen in the free space represented 44, 60, and 77% of the total oxygen per can at 40, 70, and 90° F., respectively, because 5 ml. was added to the volume of the liquid.

tinuous boiling in vacuum during condensing. Preventing contamination of the condensed milk by air seems a practical way to obtain a low oxygen level in the cans. Carbon dioxide was selected because it has the lowest oxygen content (0.005%) of the gases available.

The milk used for these experiments was either unhomogenized whole milk preheated at 194° F. for ½ hour or skimmilk preheated at 145° F. for ½ hour. Both milks were obtained from the University Creamery.

Milk was condensed in a round-bottom flask heated by hot water and kept under a pressure of 3 cm. of mercury. The total solids level was controlled by the amount of water removed, which was condensed quantitatively and received in a graduated flask. The round-bottom flask was connected to a measuring flask calibrated at 386 ml. (= 14½ oz. evaporated milk). The measuring flask was connected to a desiccator by lines of glass and tygon tubing. Stopcocks were arranged in such a way that cans in the desiccator could be filled in CO_2 atmosphere. A pressure of 10 cm. of water above atmospheric pressure was maintained by a manostat-safety valve. Open cans were placed in a circle in the desiccator. The filling line could be rotated for filling the cans one by one. At the start of an experiment, the system (measuring flask + desiccator + lines) was evacuated to a pressure of 3 cm. of mercury and filled with CO_2 ten times in succession. Immediately after the condensing, the vacuum in the round-bottom flask was broken with CO_2. The milk was transferred by CO_2 pressure via the measuring flask into the cans, which were then sealed through a small hole in the lid of the desiccator while CO_2 was continually flowing out.

Determination of oxygen, and sterilization, were as described previously.

RESULTS AND DISCUSSION

Removal of oxygen via enzymatic reaction. Results obtained at optimum incubation temperature (90° F.) are presented (Figure 1). Incubation for two hours followed by sterilization lowered the oxygen of the samples to 0.005-ml. (Curve S). However, much more oxygen was present before sterilization (Curve U); it was largely used up during sterilization. Incubation for six hours lowered the oxygen of unsterilized samples to 0.008-ml. (Curve U). Oxygen was further reduced by holding cans at 40° F. (Curve R).

Results from incubation below optimum temperature, at 70° F. and 40° F., are presented (Figure 2). The oxygen levels in unsterilized cans, that had been incubated for equal periods at 90, 70, and 40° F. (Figure 1, Curve U; Figure 2, Curves H and L), were closer together than would be expected from the changes in velocity of the enzymatic reaction with changes in temperature. It is apparent that the rate of transfer of oxygen from the free space into the milk (dissolved oxygen) was a limiting factor at the higher temperature of incubation. As the oxygen is used by the enzymatic reaction, the concentration of dissolved oxygen is lowered and, in order to maintain the equilibrium, the free-space oxygen goes into solution. The solubility of oxygen increases as the temperature is lowered. Decreased velocity of the glucose-oxidase reaction by incubation at lower tem-

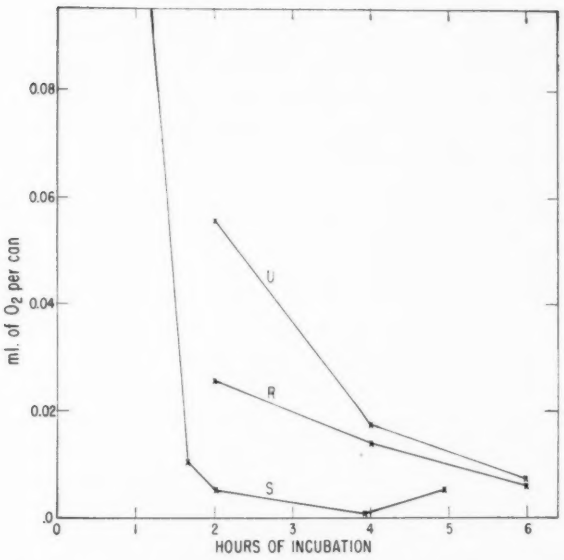

FIG. 1. Removal of oxygen from evaporated milk packed in nitrogen by incubation with glucose-oxidase at 90° F. under continous agitation. U = unsterilized, oxygen determined immediately after incubation. R = unsterilized, cans held for one day at 40° F. without agitation after incubation, then oxygen determined. S = sterilized after incubation, cans held for one day at 40° F. without agitation after sterilization, then oxygen determined.

perature is compensated by higher concentration of the dissolved oxygen, which is one of the reactants. This makes possible the removal of oxygen from evaporated milk to levels below the accuracy of determination, by incubating at below-optimum temperature (40° F.) for a long period. This method does not seriously affect quality and heat stability.

At 40° F., incubation for one day reduced oxygen to about 0.002-ml. per can (Figure 2, Curve L), which is in the range of the accuracy of determination. Yet, results from several determinations were all higher than the blank value, thus indicating that a trace of oxygen was still present. This was supported by the fact that oxygen was reduced by sterilization. Oxygen values after sterilization were between those for unsterilized samples and the blank value. Incubation for 45 hr. produced zero oxygen, and the average of several determinations was the same as the blank value. At 40° F., results were about the same for nitrogen and air-packed evaporated milk (Figure 2, Curves L and LA). Continuous agitation of the cans during incubation was necessary. Results were considerably higher if there was no agitation, or if cans were agitated during only a limited time at the start or finish of the incubation period (Figure 2, Curves HL, P, N). These variations were tried because limited agitation would be more practical for industrial application. HL samples were incubated for a

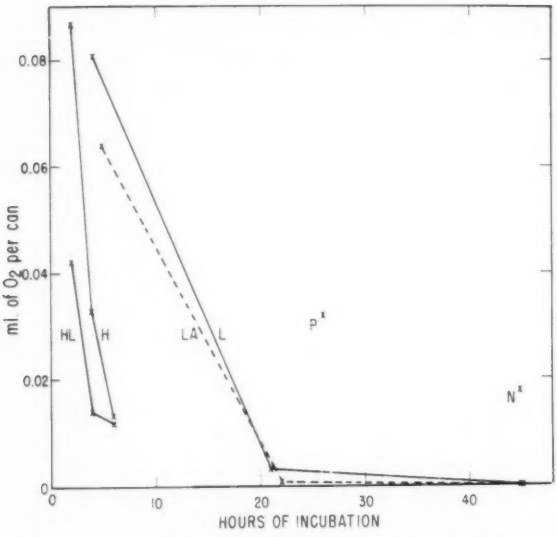

FIG. 2. Removal of oxygen by incubation with glucose-oxidase at 70° F. or 40° F. LA samples were packed in air, all others in nitrogen atmosphere. Unsterilized samples. H = incubation at 70° F. under continuous agitation, oxygen determined immediately after incubation. HL = incubation at 70° F. under continuous agitation, cans held for one day at 40° F. without agitation after incubation, then oxygen determined. L and LA = incubation at 40° F. under continuous agitation, oxygen determined immediately after incubation. P = can with glucose-oxidase held for 21 hr. at 40° F. without agitation, then agitated during five hours, then oxygen determined immediately. N = can with glucose-oxidase held for 46 hr. at 40° F. without agitation, then oxygen determined immediately.

limited time at 70° F. under continuous agitation, then held for 24 hr. at 40° F. without agitation.

If the period at 40° F. is also considered as incubation, then Curve HL has to be shifted 24 hr. to the right (Figure 2). HL data in this case are for samples incubated at 40° F. without agitation, except for a limited time at the start of the incubation period. The effect of agitation is found by comparing Curve HL with Curve L, which shows samples incubated at 40° F. under continuous agitation. Shifting of Curve HL 24 hr. to the right on the graph indicates that the samples of the HL data are considerably higher in oxygen than those of the L data. It would be still higher if the limited agitation period at the start had been at 40° F. instead of at 70° F., which is in the optimum range. Direct comparison of Points P and N with Curve L similarly shows the effect of agitation. Oxygen values were considerably higher for limited agitation at the incubation period (P) or without agitation (N). Oxygen in air-packed samples incubated for 45 hr. at 40° F., without agitation, or agitated finally for an additional five-hour period at 40° F., was 2.27 and 0.70-ml., respectively. Both values are much higher and out of range (Figure 2), as

compared with the zero value of the sample (Curve LA) incubated at the same temperature and for the same time but with continuous agitation. Results from air packing, agitation, and incubation temperature all indicated the solution rate of oxygen from the free space into the milk was a limiting factor for removal of oxygen by glucose-oxidase.

Keeping the condensed milk in carbon dioxide during processing. By this procedure, oxygen content of evaporated milk can also be reduced to minute quantities. The average content before sterilization was 0.004-ml. per can and less than 0.002-ml. after sterilization. The pH of milk processed in carbon dioxide atmosphere was consistently about 0.02-unit lower than that of the control. This value would be within experimental error of determination, if it were not for the fact that it was always negative.

Effect on color and flavor. Previous studies *(5)* have shown that lowering oxygen content of evaporated milk to from 0.2 to 0.1 ml. per can by packing the milk in nitrogen atmosphere results in a slight improvement in color and flavor. This was confirmed in the present study by organoleptic evaluation of flavor and visual comparison of color by a panel of experienced judges. Further lowering (as low as 0.002-ml. per can) of the oxygen level, as was accomplished by the use of glucose-oxidase, did not produce additional improvement in either color or flavor. It is probable that adding glucose as the substrate for glucose-oxidase accelerated the browning reaction, thus minimizing the effect of extremely low oxygen concentration. The most significant improvement was obtained by elimination of oxygen, by processing and packing in carbon dioxide atmosphere. Differences in color and flavor from the controls were maintained during six months of storage.

SUMMARY

The method of Pepkowitz and Shirley *(3)* was modified for determination of low concentrations of oxygen (from 0.1- to 0.001-ml. per can) in evaporated milk.

A procedure was outlined for the removal of oxygen with glucose-oxidase, by incubating at low temperature for a long period of time (from 24 to 48 hr.) under continuous agitation of the cans. Slow transfer of oxygen from free space into the milk was a limiting factor at higher temperature of incubation and at low levels of oxygen. Oxygen was reduced below 0.002-ml. (S.P.T.) per can of evaporated milk (0.03% of the total original oxygen) with glucose oxidase and incubation at 40° F. for 40 hr. with continous agitation of milk. This is in the range of the accuracy of determination. Levels of 0.004-ml. per can were obtained by processing the condensed milk in an atmosphere of carbon dioxide.

Lowering of oxygen to these extremely low levels did not materially affect the browning of milk by sterilization and storage. This indicates that browning can proceed, even though only traces of oxygen are present—perhaps through the channels that do not require oxygen *(2)*.

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INFLUENCE OF LIPOPROTEIN ON THE FREEZING OF BOVINE SPERMATOZOA[1,2]

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The effects of lipoprotein on the survival and livability of spermatozoa, frozen at low temperatures, were studied. Five per cent added lipoprotein increased sperm survival about 5%, a 2.5% level was ineffective, and the 10% was no better than the 5% level. Differences in percentage of live sperms and in motilities between the control and 5% added lipoprotein were highly significant in favor of the 5% level. *Editor.*

One of the more important problems presented by the discovery of a successful method for prolonging the life of mammalian spermatozoa has been the development of a medium that would protect them from adverse environmental changes during freezing.

Since the introduction of the egg yolk diluent, studies have been under way to isolate the factor or factors in egg yolk which benefit spermatozoan livability. Lasley and Mayer *(5)* and Mayer and Lasley *(7)* isoloted a white crystalline substance from egg yolk, which they found very effective in protecting spermatozoa from cold-shock. Bogart and Mayer *(1)*, in a subsequent study, showed that this substance protected stallion spermatozoa from cold-shock and from changes in pH, osmotic pressure, and the possible accumulation of harmful substances.

Recently, Kampschmidt and coworkers *(3)* studied the lipoprotein and lipid constituents of egg yolk and their relation to the resistance and storage of bovine spermatozoa. They found that egg yolk lipoproteins protected bovine spermatozoa from cold-shock and were also beneficial as a storage medium. Egg yolk phospholipids protected spermatozoa from cold-shock, but they gave poor results as storage media. Kok *(4)* also stated that egg yolk lipoprotein prolonged the life of spermatozoa and protected them from the adverse effects of cooling, but that it is perishable and of variable quality.

On the basis of available information, an experiment was designed to study the effect of added lipoproteins on freezing bovine spermatozoa.

EXPERIMENTAL PROCEDURE

Lipoproteins used in this study were isolated by methods similar to those described by Kampschmidt *(3)* and Chargaff *(2)*. The method employed was

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[1] These data were taken from material submitted by the senior author in partial fulfillment of the requirements for the Master of Science degree, The Ohio State University, Columbus, Ohio, 1954.

[2] The study was conducted in cooperation with the Central Ohio Breeding Association, Richard Kellogg, Manager.

[3] Present address: Department of Dairy Husbandry, University of Wisconsin, Madison, Wisconsin.

as follows: Five egg yolks were thoroughly mixed with 75 ml. of saturated NaCl solution. This emulsion was extracted four times at two-hour intervals with 150 ml. of moisture-free C.P. ethyl ether. Following the last ether extraction, the extracted egg yolk–NaCl mixture was poured into 25 volumes of cold distilled water and stored overnight in a refrigerator. The following morning the precipitated fraction was separated from the supernatant and washed three times with 50-ml. portions of ethyl ether.

The purpose of the ether extractions was to remove the ether-soluble lipids. The purified lipoprotein fraction was then dissolved in 10% NaCl solution and subsequently poured into six volumes of cold distilled water. The white lipoprotein flocculent was separated by centrifugation in a refrigerated centrifuge and then washed six to eight times with cold distilled water. The isolated lipoproteins were dispersed in 2.9% sodium citrate buffer at the ratio of 1 g. of lipoproteins to 10 ml. of the buffer and then stored at −20° C.

During the first phase of the experiment, the effect of three different levels of added lipoprotein was compared with the control to which no lipoprotin was added. The levels of added lipoproteins solution comprised 2.5, 5, and 10% of the total semen dilution. Fifteen samples were treated and examined during this phase of the work. The last phase of the study consisted of a split-sample trial, during which 32 semen samples were frozen. Only the 5% level of added lipoproteins was tested, since the previous phase of work indicated this level to be the most beneficial.

The freezing procedure employed was similar to that of Macpherson and Henderson *(6)*. The final glycerol concentration was 7.5% by volume. The samples were equilibrated with glycerol at + 5° C. for from 14 to 18 hr. The freezing rates were as follows: +5 to − 10° C., 1° C. every three minutes; − 10 to − 15° C., 1° C. per minute, and − 15 to − 75° C., 6° C. per minute. The last temperature drop was accomplished by adding large quantities of crushed dry ice to the freezing tray. Samples were stored in alcohol in a mechanical deep-freeze at − 96° C. Examination of the frozen samples was performed immediately after freezing and after storage for two, nine, 16, and 23 days in the deep-freeze. The samples were thawed in + 5° C. water and examined under microscope for the per cent of motile spermatozoa and for the rate of motility. Motility scores ranged from zero (sample dead) to ten (excellent rate of motility). Microscopic evaluation of six fields was made for each sample and averages of these readings were used as a final rating.

Results were analyzed by the t test of a mean difference for paired comparisons *(8)*. The homogeneity of variances was tested by Bartlett's method *(8)*.

DISCUSSION AND RESULTS

Averages of the 15 treated and 15 control samples are shown (Table 1). The computed t-values for all possible comparisons of the means are shown (Table 2). (The t-value for $P = 0.01$ as indicated in the footnote to Table 2, is 2.977.) Consideration of the data in this table would tend to indicate that

the addition of small amounts of lipoproteins (less than 2.5%) does not increase resistance of bovine spermatozoa to cold-shock during the freezing process. There is some indication, however, that the addition of 5% lipoproteins has a definite benefit on sperm quality. This improvement is indicated to a higher degree in the evaluation of per cent live cells than in the motility ratings.

Data for the 32 semen samples, in which the effect of only the 5% level of added lipoprotein was compared to the control, are represented (Table 3). The t-value for the difference in the per cent of live spermatozoa was 8.79 and the t-value for the difference in motility rating was 5.4. The value of t .01:31

TABLE 1

Averages of per cent of live cells and of motility ratings of 15 bovine semen samples frozen with four liproprotein levels

Per cent live cells					Motility rating				
		Lipoprotein added					Lipoprotein added		
Sample	Control	2.5%	5%	10%	Sample	Control	2.5%	5%	10%
1	44	41	53	45	1	7.0	6.8	7.4	6.6
2	54	50	62	53	2	7.0	7.0	8.0	7.4
3	22	20	25	25	3	5.0	5.2	5.6	5.6
4	35	36	41	40	4	7.2	7.0	7.6	7.6
5	53	55	57	56	5	6.8	6.8	7.4	7.4
6	20	22	29	23	6	4.6	4.8	5.0	4.8
7	30	31	34	35	7	6.5	6.5	6.8	7.0
8	47	52	54	55	8	7.0	7.3	7.8	7.8
9	55	56	64	62	9	7.8	7.8	9.0	8.4
10	33	33	38	40	10	6.8	6.6	6.8	7.0
11	60	60	68	63	11	8.6	8.4	9.2	9.2
12	38	40	47	43	12	7.4	7.4	8.2	7.6
13	6	8	11	9	13	5.0	5.0	5.2	5.2
14	51	51	57	59	14	7.2	7.2	7.4	7.6
15	36	35	44	41	15	7.2	7.0	8.0	7.4
Av.	39	39	46	43	Av.	6.7	6.7	7.3	7.1

TABLE 2

Paired t-test of a mean difference in the per cent of live spermatozoa and of motility ratings of 15 samples frozen with three levels of lipoprotein

	Comparison of treatments					
t – value[a]	Control and 2.5%[b]	Control and 5%[b]	Control and 10%[b]	2.5% and 5%	2.5% and 10%	5% and 10%
t – per cent live cells	0.72	11.68	5.46	4.54	6.55	3.92
t – motility	0.5	6.548	4.8	6.786	5.91	1.579

[a] —$t(.01:14) = 2.977$.

[b] —Level of added lipoproteins.

TABLE 3

Average evaluation of 32 semen samples frozen with and without lipoproteins

Criteria for evaluation	Control	5% added lipoprotein
Per cent live cells	25.2	30.5
Motility rating	5.4	5.9

is 2.744. From the examination of the available literature dealing with the physiological and chemical properties of lipoproteins, it is difficult to advance any single explanation of the results obtained. Since we know that the egg yolk lipoproteins exert a beneficial effect on the livability of unfrozen spermatozoa, it is possible that this beneficial action is maintained in the frozen state also.

It is also reasonable to hypothesize that in whole egg yolk the lipoprotein component is combined with other egg yolk constitutents and thus can not exert its full beneficial action. Two possible sites of lipoprotein activity could be the cell membrane or the protoplasm. It has been suggested that the phospholipid fraction of the lipoprotein molecule can regulate the permeability of the cell membrane and be helpful in the maintenance of the protoplasmic structure, which is subjected to many stresses during freezing and thawing.

Many more explanations could be advanced, but their relationship would be difficult to explain. More work is needed on the problem of cell survival in subzero temperatures, before the exact nature of the lipoprotein function can be ascertained.

SUMMARY AND CONCLUSIONS

An experiment was designed to study the effect of added lipoproteins on the revival of deep-frozen spermatozoa. Forty-seven semen samples were subjected to the lipoprotein treatment. Under the conditions of the experiment, a 5% level of added lipoprotein increased the sperm survival rate approximately five percentage units. A 2.5% level was wholly ineffective, whereas a 10% level was no better than the 5% level. The differences in the per cent of live spermatozoa and in motility rating between the control and the 5% level of added lipoproteins were, statistically, highly significant in favor of the 5% level. Whether these differences will have a beneficial effect on the fertilizing capacity of the treated semen samples, can not be elicited from this study.

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OBSERVATIONS ON THE NUMBERS OF VIRUS-RESISTANT MUTANTS DEVELOPING IN THE HOST-VIRUS SYSTEM OF THE LACTIC STREPTOCOCCI [1, 2, 3]

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Virus-resistant mutants in single-strain cultures of lactic streptococci were studied, to determine the effects of temperature and age of culture on mutation rate, using two plate culture methods and a multiple subculture method. The number of resistant mutants by plating methods varied from 0 to 66.2 per 1×10^6 colony count of the test cultures. The mutation rate of two Streptococcus lactis cultures was higher than that of four cultures of Streptococcus cremoris. The mutation rate of one strain of S. cremoris was so low it could not be measured by plate culture methods, but the subculture method revealed a resistant mutant ratio of about 1 per 3×10^6 host colonies. *Editor.*

Cultures of the lactic streptococci, when attacked by bacterial virus, usually recover their activity during subsequent incubation, because virus-resistant strains develop. Luria and Delbruck *(3)* were the first to show that virus-resistant strains are mutants of the susceptible host cells. They further showed that this phenomenon is not induced by the virus, but is entirely independent of its presence. Newcombe *(4)*, likewise, showed that virus-resistant variants arise by spontaneous change before contact with the virus.

The study reported in this paper was made to determine the extent to which virus-resistant mutants occurred in several single-strain cultures of lactic streptococci, and to ascertain the influence of incubation temperature and culture age on the mutation rate.

METHODS

Cultures and virus strains. Six single-strain cultures and their homologous virus strains were used in this study. With the exception of the HP strain obtained in 1946 from Dr. H. R. Whitehead of The Dairy Research Institute, New Zealand, the cultures and virus strains were isolated from dairy plants having difficulty in maintaining satisfactory starter activity. Two were strains of *Streptococcus lactis* and four were strains of *Streptococcus cremoris.*

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[2] This investigation was supported in part by funds provided for Biological and Medical Research by the State of Washington, Initiative Measure No. 171.

[3] These data were taken in part from a thesis presented by the junior author to the faculty of the State College of Washington, to partly fulfill the requirements for the M.S. degree.

Virus concentrates. Virus concentrates were prepared in the form of tomato juice broth filtrates. The composition of the tomato juice broth was: filtered tomato juice, 400 ml.; water, 600 ml.; proteose peptone, 1%; peptonized milk, 1%; and $CaCl_2$, 0.03%. $CaCl_2$ was included in the medium to enhance the proliferation of virus, as shown by Potter and Nelson *(5)*.

Bromeresol purple indicator was added at the rate of 8 ml. of a 0.4% solution per liter of broth. The pH of the medium was adjusted to 6.6 before sterilization. Two 250-ml. portions of the broth were inoculated at the 4% level with a 24-hour-old broth culture of the test organisms, and then incubated at 30° C. At the first evidence of turbidity, one broth culture was inoculated with the homologous-virus concentrate at the 4% level.

As the cultures developed, periodic transfers of approximately 25 ml. each were made from the virus-free culture to the one containing virus. This transfer provided large numbers of susceptible host cells for virus proliferation. Care was taken to maintain the approximate initial pH of the two cultures during incubation. An alkali solution was added, to correct pH shifts indicated by the color change of the medium.

After six to eight additions of the susceptible host cells had been made, the lysed bacterial suspension was passed through a sterile Seitz filter. The filtrate was stored at from 0 to 5° C. until used. This procedure readily gave a virus concentrate of high titer.

Enumeration of virus-resistant mutants. Plating methods. Plating procedures involved exposing appropriate serial dilutions of the culture to homologous virus in a suitable medium, followed by lysing of the susceptible cells and growth of the resistant strains into colonies during the incubation period. Two methods of preparing plate cultures were used in this study:

Method 1. Serial dilution pour plates. Nine-ml. amounts of the virus concentrate were aseptically dispensed into sterile screw-capped tubes of 20-ml. capacity. Using this concentrate as a diluting medium, serial dilutions were prepared through the range of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. Following an adsorption period of five minutes at 30° C., tomato juice agar plate cultures were made in duplicate, incubated at 30° C., and the colonies counted after 48 hr.

Method 2. Serial dilution surface plates. Luria and Delbruck *(3)* and Demerec and Fano *(2)*, using a culture of *Escherichia coli*, first spread the virus concentrate over the surface of the plate and then spread the test culture over the same area. Beale *(1)* and Newcombe *(4)*, also using *E. coli*, spread the test culture over the surface of the medium and then applied the homologous virus concentrate in the form of an aerosol. The technique used in this study was a modification of that used by Newcombe.

Serial dilutions, using a sterile 0.3% $CaCl_2$ solution as the diluting medium, were made as in Method 1. One-tenth ml. portions of the respective dilutions were spread with a glass rod over the central area of a poured and surface-dried tomato juice agar plate, leaving a margin of at least 1 cm. Following a period of several minutes, to permit the surface of the agar to dry, the virus concentrate was applied over the entire surface of the plate as an aersol,

by means of a hand-operated atomizer. A sterile filter paper disc was placed in the lid of the petri dish to absorb the excess moisture. Incubation and the counting of colonies were the same as in Method 1.

Multiple subculture method. The number of virus-resistant mutants in one host culture proved to be too few for enumeration by either plate method. To obtain data for this strain, it was cultured in milk to the point of coagulation, after which multiple subcultures of 1 ml. each were made into 100-ml. portions of sterile milk. Except for a control, all subcultures were inoculated with 1 -ml. portions of the homologous virus concentrate; the contents were thoroughly mixed and incubated at 30° C. The cultures were observed for ten days. Those showing evidence of growth were subcultured in the presence of additional homologous virus concentrate for confirmation of their virus-resistant characteristic.

Total count. Simultaneously with the enumeration of the virus-resistant mutants, the total colony count of the respective cultures was made by the plate method, using tomato juice agar. The cultures were incubated at 30° C. for 48 hr.

RESULTS AND DISCUSSION

Calculations were made on the basis of the number of virus-resistant mutants per one million colonies of the test culture. The figure obtained is referred to as the mutation rate.

Results using Method 1 are shown (Table 1).

TABLE 1

Virus-resistant mutants in cultures of lactic streptococci — effect of temperature of incubation and age of culture

(Number per million colonies of culture)[a]

	Temperature of incubation					
	20° C.		25° C.		30° C.	
	Age in hours		Age in hours		Age in hours	
Culture	6	24	6	24	6	24
S. cremoris—#6	1.8	2.5	3.4	2.6	2.9	3.2
S. cremoris—X	6.3	7.7	4.9	4.8	3.0	3.3
S. cremoris—HP	1.2	1.6	1.1	1.1	1.3	1.3
S. cremoris—W	0.0	0.0	0.0	0.0	0.0	0.0
S. lactis—Hbs	51.2	33.1	44.7	39.0	66.2	33.8
S. lactis—St	15.8	4.9	12.1	4.7	10.7	6.3

[a] Logarithmic average of ten trials. Figures referred to as "mutation rate."

The mutation rate ranged from 0 to 66.2. The cultures of *S. cremoris* showed a lower mutation rate than did the cultures of *S. lactis*. Owing to the limited number of cultures of *S. lactis* used in this study, no significance can be attached to this difference.

Among the cultures of *S. cremoris*, few or no differences in mutation rate were associated with either the temperature or the age of the culture. The younger cultures of the two strains of *S. lactis*, in every instance, showed a slightly higher mutation rate than did the older cultures. The older cultures, in every case, were coagulated. In this condition, these cultures were not so suited for the enumeration of bacterial numbers by the plate culture method

as were the younger noncoagulated cultures. The differences noted are probably more apparent than real.

Preliminary efforts often failed to obtain consistent distribution of colonies among the four serial dilution plate cultures. Frequently, plate cultures of the 10^{-4} dilution showed more colonies than plate cultures of the lower dilutions. This discrepancy was found to be associated with relatively low concentrations of virus particles in the dilution medium, a condition which apparently diminished the opportunity for contact of virus particles and susceptible cells. After this had been established, virus concentrates having a titer of less than 10^8 were not used as diluting media.

A comparison of Methods 1 and 2 is presented (Table 2).

TABLE 2

Comparison of Methods 1 and 2 for enumerating the virus-resistant mutants in 24-hour-old cultures incubated at 30° C.
(Numbers per million colonies of culture)[a]

Culture	Method 1	Method 2
S. cremoris—#6	3.2	7.7
S. cremoris—X	3.3	3.3
S. cremoris—HP	1.3	3.1
S. cremoris—W	0.0	0.0
S. lactis—St	6.3	14.5

[a] Log average of ten trials.

Satisfactory agreement was obtained betwen Methods 1 and 2. The slightly higher mutation rates obtained by the use of Method 2 are well within the range of variation inherently associated with the plate culture method. In Table 2 no comparison is shown for the Hbs strain of *S. lactis*. During the interval between these two experiments a pronounced change occurred in this strain; the mutation rate increased many fold.

Tables 1 and 2 disclose that the W strain of *S. cremoris* showed a mutation rate of 0. The small inoculum, which it was necessary to use in these plating methods, failed to result in the development of any resistant mutant strains. Accordingly, an experiment was designed for use of larger inocula and on a multiple basis. This is referred to as the multiple subculture method. Results of a series of observations are presented (Table 3).

TABLE 3

Virus-resistant mutants from W strain S. cremoris
(Multiple subculture method)

Trial	No. of 1.0 ml. subcultures	Subcultures positive for mutants	Total colony count of multiple inocula
			(millions)
1	100	1	70,000
2	100	2	57,000
3	50	1	29,000
4	100	4	60,000
5	100	2	65,000
6	100	1	48,000

In the six series, using Strain W, a total of 550 subcultures were made in which the host cultures were exposed to the presence of the homologous virus. Eleven subcultures developed to the point of coagulation. On subsequent examination, each revealed the presence of virus-resistant strains. Calculated on the basis of the total colony count, the ratio of the resistant mutants was about 1 per 30,000,000,000 host colonies.

SUMMARY

Two plate culture methods and a multiple subculture method were used to determine the numbers of virus-resistant mutants present in six single-strain cultures of lactic streptococci. The number of resistant mutants, as determined by the plating methods, ranged from 0 to 66.2 per 1,000,000 colony count of the test cultures. The mutation rate of the two *Streptococcus lactis* cultures was higher than that of the four cultures of *Streptococcus cremoris*.

The mutation rate of one strain of *S. cremoris* was too low to be measured by the plate culture methods. By means of a multiple subculture method, the ratio of resistant mutants was found to be about 1 per 30,000,0000,000 host colonies.

The age of the culture, six and 24 hr., and the temperature of incubation, 20, 25, and 30° C., appeared to have little effect on the mutation rate.

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TECHNICAL NOTES

HEMOGLOBIN VARIANTS IN DAIRY CATTLE

A number of hemoglobin variants have been known and studied in humans for some time *(5)*, but only recently has the attention of investigators turned to the various species of livestock. In the past year, Evans *et al. (4)* have reported a hemoglobin variant in sheep, and still more recently, Bangham *(1)* has reported electrophoretically different hemoglobins in certain cattle breeds of Great Britain. Prior to the publication of his paper, these observations were made known to the authors by personal communication. Because the origins of our breeds of cattle are in common with those of Europe, and since no study of this nature had been reported in the United States, it seemed desirable to investigate the possible existence of hemoglobin variants in our American cattle.

As a source of preliminary information, animals in the purebred and crossbred herds of the University of Illinois were tested. Because animals less than three months of age gave undefinable results, our study was restricted to those that were older. This lack of definition will be discussed subsequently. A sample of approximately 5 c.c. heparinized venous blood was drawn from each animal tested. The erythrocytes were separated from the plasma by centrifugation. The cells were washed in 0.9% sodium chloride and hemolyzed with a quantity of water sufficient to give a hemoglobin concentration of approximately 10% by volume.

Electrophoretic separation was accomplished on a Durrum type (Spinco) apparatus *(2)*. Ten microliters of each 10% hemoglobin solution was placed upon a Whatman filter paper strip. Analyses were made in veronal buffer, pH 8.6, for 3 hr. at 15 v. per cm. The strips were then dyed in bromphenol blue, washed in 5% acetic acid, and fixed in acetate buffer. Results were determined by visual examination.

This technique made possible the description of two hemoglobins with distinct, well-defined differences in isoelectric point. One type, which appears to be most common, is characterized by a relatively slow rate of mobility. This was designated Type A. The second type, designated B, exhibits a more rapid rate of mobility. The two types may occur either separately or in combination. When both types occur in the blood of one animal, analysis shows distinct bands of both A and B on the paper strip. This result has been denoted AB. It has also been demonstrated that when homogenous blood of Type A is mixed with homogenous B, the above observation can be duplicated.

Results obtained from study of 125 animals representing four breeds are shown (Table 1). It will be noted that Holsteins have only Type A; Guernseys and Brown Swiss both types, with A predominating, and the Jerseys both types, with B predominating. It is believed that this analysis of hemoglobins in Brown Swiss is the first reported for this breed. The other data agree fundamentally with the observations of Bangham.

TABLE 1

Breed	Number tested	Hemoglobin type A	AB	B
Holstein	17	17	—	—
Guernsey	34	27	7	—
Brown Swiss	14	12	2	—
Jersey	11	1	6	4
Crossbred				
¾ Holstein— ¼ Guernsey	10	10	—	—
½ Holstein— ½ Guernsey	26	23	3	—
¼ Holstein— ¾ Guernsey	13	13	—	—
Total	125	103	18	4

Although the genetic study is as yet incomplete, evidence obtained thus far supports Bangham's theory on the inheritance of the character. Presumably, it is controlled by an allelic pair of genes at a single locus, with neither allele exhibiting dominance. We propose to designate these genes as HbA and HbB, controlling bovine hemoglobin Types A and B, respectively. This provides a nomenclature for reference to both the hemoglobin type and the specific gene associated with it. Thus, an animal homozygous HbAA would show the electrophoretic result A; the homozygous HbBB, the result B, and the heterozygote HbAB, the double band which we have called AB.

Bangham pointed out confusing results when animals less than three months of age were tested by this technique. Our study of a total of ten Holstein, Guernsey, and Holstein-Guernsey crossbred calves showed that in animals less than 80 days of age, a third hemoglobin variant was invariably present. This variant was characterized by a broad, diffuse band, which indicated a range of mobility encompassing the rates of both A and B, although the major concentration appeared to be in the B range of mobility. It was noted that this component predominated at birth but that its magnitude declined steadily with age until it disappeared at approximately 80 days. This is closely associated with the known presence at birth of "fetal" erythrocytes of lymphatic origin and the normal rate of destruction of erythrocytes *(3)*. It seems logical to assume, therefore, that this third hemoglobin variant is produced in the lymphatic tissue and is present in the fetal erythrocytes. Pending further investigation, this variant has been tentatively designated fetal hemoglobin, or Type F.

The observations of Bangham have been essentially duplicated, and the existence of hemo-

globin variants in mature animals of at least three breeds of dairy cattle in the United States has been established. It is noteworthy that in humans all of the hemoglobins thus far described other than Type A are abnormal, and have been associated wth some deleterious or pathogenic effect *(5)*. Although at this time our data are quite insufficient to support such an hypothesis for cattle, it is interesting to speculate as to the possible effects of hemoglobin Type B upon the animal which carries it in the homozygous state. Investigations are being made in this regard as the study is continued.

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DETECTION OF 1-MONOGLYCERIDES IN THE FAT FROM RANCID MILK

Recent work indicates that pancreatic lipase hydrolyzes triglycerides to 1,2-diglycerides and then to 2-monoglycerides *(1, 2)*. The 1-monoglycerides isolated in these studies are believed to result from isomerization of the 2-monoglycerides *(1)*. Assuming that milk lipases act in the same manner, these partial glycerides should be present in the fat of rancid milk.

Two samples (900 ml. and 2,700 ml.) of rancid milk, prepared by mixing raw and homogenized milk in equal portions, were extracted with Mojonnier reagents and the solvents evaporated. The fat thus obtained was partitioned between heptane and 80% ethanol, as described by Borgstrom *(3)*. The 1-monoglyceride contents were determined by the periodic acid oxidation method described by Martin *(5)* and are listed (Table 1). A factor of 298, based upon a butterfat saponification value of 236, was used in the calculation of the monoglyceride content.

Attempts to increase further the 1-monoglyceride content of the 80% ethanol fractions by chromatographic fractionation on silicic acid columns, with chloroform containing varying amounts of methanol, were not successful.

TABLE 1
The 1-monoglyceride content of fat fractions from rancid milk

Fraction	Sample	Weight	1-monoglycerides
	(*No.*)	(*gm.*)	(%)
Extracted fat	1	25.1	1.07
	2	98.1	1.17
Heptane-soluble fat	1	24.4	Not run
	2	96.8	0.14
80% Ethanol-soluble fat	1	0.5196	60.48
	2	1.0915	54.17

The infrared absorption spectra of the lipid fractions and monopalmitin were measured in anhydrous alcohol-free chloroform. The 80% ethanol-soluble fractions showed characteristic absorption maxima at 3.0, 9.5, and from 10.1 to 10.2 microns, indicating the presence of monoglycerides (6). The spectra of the extracted fat and the heptane fractions revealed that both extracts contained only small amounts of monoglycerides.

The detection of monoglycerides in rancid milk fat introduces several interesting facets to the problem of rancidity. Monoglycerides are known to be foam-depressants and fat-emulsifiers. Brunner *(4)* was able to reduce the foaming of skimmilk in a vacuum pan by adding monoglycerides. These compounds are surface-active and, hence, may have some disrupting effect on the milk fat globule membrane. They should also effect the detection of rancidity by measurement of surface-tension reduction. Further study with the present methods, in conjunction with methods for diglycerides *(2)*, may reveal the mode of triglyceride hydrolysis by milk lipases.

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OUR INDUSTRY TODAY

Brief Reviews of Current Topics

Problems Associated with Production Testing and Pipeline Milkers

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A survey in North Carolina in 1954 showed that 7.5% of the farms producing Grade A milk were using pipeline milkers. This number had increased to 18% early in 1957. This trend is probably even greater in some of the other states. The development of the pipeline milker for the stanchion barn as well as for the milking parlor is a step toward greater labor efficiency and milking convenience in our modern dairy farms. From all indications, the use of the pipeline milker is going to continue to grow. While the pipeline milker is making the job of milking cows easier, it is creating new problems in other areas.

This system of milking cows does not lend itself to easy weighing and sampling of each cow's milk, which is so necessary for production testing. Consequently, many dairymen are struggling with the decision either of making their job easier by ignoring some of the recommended management aids, such as production records, or, of using a system for milking that is not as convenient, requiring more labor to enable them to keep production records.

For more than half a century, the keeping of production records on individual cows has been recognized as a desirable practice for the improvement of our dairy cattle. At the present, it is fast becoming a requirement in dairy herd management, if dairy farmers are to improve their average production per cow and maintain a level of production that will help them compete with the many costs now associated with dairy farming. But production testing is not being practiced on many dairy farms as yet, for one reason or another. And now the development of the pipeline milker, transporting milk direct from the cow to the bulk tank, has become another reason to prevent some dairymen from keeping production records. Should this be a serious disadvantage to keeping production records on individual cows?

Pipeline Milkers vs. Production Records

Some dairymen with pipeline milkers have already discontinued production testing until something more convenient is developed for weighing and sampling each cow's milk. Others realize the importance of keeping production records for herd management, and have adopted various systems for getting individual milk weights and samples for each cow. A few of these systems are good, but some are far from being desirable. In some instances, accuracy has been sacrificed for convenience. Changes in milking routine on test day have resulted in irregularities in weights and tests. Unusual variations have caused a few to lose confidence in production records. Some systems are time-consuming, causing delay in milking, with excessive equipment to clean up. The extra equipment used in many instances is expensive and breakable. These are some of the problems we have to recognize, if production testing is to continue to expand and keep pace with our modern systems of housing and milking dairy cows.

It should be pointed out that dairy farmers using pipeline milkers need production testing more than anyone else. In this system, they never have an opportunity to see how full, or to feel how heavy, the milker pail may be when milking each cow. Even this is no substitute for the actual weighing and sampling of each cow's milk, although it is often used by those who think they know their cows (without keeping production records). Therefore, we need a system to weigh and sample each cow's milk, used with pipeline milkers, that will save time and labor and be accurate, convenient, and inexpensive. Considerable work is being done on this problem and there are several ways that each cow's milk can be weighed and sampled along with use of the pipeline milker. Very few, if any, of the systems meet all of the desired qualifications, but some systems are better than others.

Use Old Milker Pails

The simplest, and probably the surest way, to get an accurate milk weight and sample from each cow, is to use the old milker pails on the day the tester is at the farm. The milk is poured from the milker pail to the tester's weigh pail, and weighing, mixing, and sampling are done in the usual manner. This is not the easiest or most convenient way, since the milk has to be carried to the milk room or drawn from the tester's weigh pail to the pipeline, requiring extra time. In spite of this inconvenience, it must be pointed out that weighing and sampling for production testing is required only one day each month—only twelve times a year.

Keeping the old milker pails, when installing a pipeline milker, is a wise idea, as they provide an inexpensive way to collect each cow's milk individually. However, too often the old milkers are traded in and the dairyman has no way to weigh and sample each cow's milk without purchasing a special device for this purpose. On the other hand, some dairymen do not like to change to the old milker pails one day a month. They claim this is annoying to the cows, resulting in abnormal weights and tests.

Holding Containers by Manufacturer

A more convenient system to weigh and sample each cow's milk is to secure special containers, provided by the manufacturer, that will fit in the pipeline system for this purpose. Some of these work rather well, whereas others leave much to be desired when accuracy is considered. They will collect each cow's milk individually, but some other things need to be considered. Some units are equipped with poor scales that do not last or stay in balance. At best, a slight change in tension of connecting hoses can cause milk weights to be high or low. To be absolutely accurate, all hoses should be disconnected from the holding container. This takes time, so accuracy is often sacrificed for convenience, by leaving the hoses connected.

Holding Jars Used for Weighing and Sampling

Some have tried to eliminate this source of error by using calibrated jars. These are even less satisfactory, and this system has not been approved for standard DHIA and official records. Care needs to be taken that holding containers are free of rinse water when the first cow is milked, and that they are drained thoroughly between milking each cow, with no milk returning from the milk line. Getting a representative sample for each cow is difficult when these holding containers are used, owing to the inability to mix the milk adequately. Most devices are arranged to let air bubble through the milk, ranging from merely replacing the vacuum in the container to drawing air through the milk for an indefinite period of time. Replacing the vacuum with air does not give enough mixing for an accurate test. In two DHIA herds where this system was used, the average herd dropped 0.5 and 1.0% in butterfat test. In the latter, individual cow comparisons were made, with the milk being drained from the container and thoroughly mixed. Twenty-one out of 23 cows had a higher test, ranging from 0.1 to 1.5%.

A study at Clemson College shows that air should be bubbled through the milk in the holding jar for one second for each pound of milk, if butterfat tests are to check with samples from milk thoroughly mixed. If the milker is in a hurry, and does not cooperate with the tester, mixing is often not adequate, especially for high-producing cows. Holding containers sometimes are too small, resulting in milk being drawn into the vacuum line, or requiring partial emptying of the container. This makes it difficult to get an accurate sample, and is inconvenient.

In some systems, both manufactured and home-made, practically no mixing of the milk can be done. The sample is taken from the holding container with a sample dipper by removing the top, or the container is tipped to one side permitting milk to flow into a special sampling chamber. A study made at the University of Maryland shows that a representative milk sample can not be obtained by taking milk direct from the milker pail without mixing. Pouring the milk from one pail to another at least twice was necessary to provide sufficient mixing to assure a representative sample.

At times, the convenience of some of this special equipment is even questioned by the tester.

Home-Made Devices

Some dairymen with pipeline milkers discontinue testing, whereas others hesitate to start, because of the cost necessary in securing additional equipment for weighing and sampl-

ing. In some instances, costs for this equipment seem rather excessive. However, being able to cull intelligently two or three low-producing, unprofitable cows could very likely save the dairyman enough money to purchase the necessary weighing and sampling equipment (which could be used for a number of years, given reasonable care). Some dairymen have not let the cost of the additional equipment stop them from keeping production records. They have kept the initial cost to a minimum by developing home-made systems for collecting each cow's milk. One DHIA member made up the holding container shown (see picture below) for a total cost of $7.77, not including the tubing. In the milking parlor this pail can sit on the floor and is not disturbing to the cows. This DHIA member lends the container

A Simple, Inexpensive Home-Made Device that Works Satisfactorily

to his neighbors, for use on test day. The tester finds this to be a very satisfactory and inexpensive way to collect each cow's milk separately. Others have secured special covers from milking-machine manufacturers, for milk cans or milker pails that will fit into the vacuum system. The milk is poured from the holding container to the tester's weigh pail and weighed and sampled in the usual manner. Home-made systems usually are less costly, but not as convenient. However, satisfactory weights and samples can be secured, if systems are properly designed.

Metering Devices

To keep pace with the convenience of the milking parlor and the pipeline milker, a metering device, referred to as the Milk-O-Meter, has been developed for weighing and sampling each cow's milk. The milk passes through this device, going from the cow to the pipeline, where it is measured in one-fourth pounds. A representative sample is taken as the milk passes through the meter. Tests to date have not shown this metering device to be entirely satisfactory for accurate weighing. Research data obtained on Milk-O-Meters, calibrated for a flow rate of from 3 to 4 lb. of milk per minute and used in 12- to 13-in. vacuum installations, shows that individual milkings of fast-milking, high-producing cows tend to be underweighed as much as 3 to 4% by this method. The reverse may be true on slow-milking, low-producing cows. Meters should be calibrated by the manufacturer to the flow rate of the herd or herds in which the meter is to be used. Even this will give individual cow variations, depending on whether they are fast or slow milkers. Improvement and further research on the weighing accuracy of this device is needed, before it receives widespread use in DHIA herds.

If a metering device can be developed to weigh milk satisfactorily when pipeline milkers are used, dairymen will probably be less inclined to discard production testing when they move to the more modern labor-saving methods in milking and handling dairy cows. However, there still will be problems involved with a metering device, such as cost, checking for accuracy, and additional equipment to clean and handle. Even though cost may remain a major disadvantage from the farmer's point of view, other problems associated with weighing and sampling milk with pipeline milkers should be minimized.

The cost of any weighing and sampling device can be kept to a minimum by distributing it among the herds on test. Some weighing and sampling equipment could become part of the standard testing equipment, owned by the testing association, and carried by the tester. A sufficient number of units would have to be purchased by the association to accommodate the herd milking the greatest number of cows at one time. In some cases, it might be desirable for the association to purchase only part of the units needed, and for the individual herd to secure additional units to use when the tester is at the farm. In the beginning, perhaps only the herds with pipeline milkers would want to purchase cooperatively the necessary weighing and sampling devices. These arrangements can be worked out locally, to provide for greater use of this equipment at a minimum investment. Weighing and sampling devices used in this manner should be made from durable material, that will stand considerable abuse and wear.

Accuracy Is Important

Accuracy is important in production testing. Careful checking is necessary, to determine the degree of accuracy of each system being used for weighing and sampling milk from individual cows. All weights and tests will not

agree when taken from different systems of weighing and sampling. We are then confronted with how much variation from conventional methods can be permitted and still have the weights and tests accurate.

In approving weighing and sampling devices for use in the National Dairy Herd Improvement Program, at its annual meeting in June, 1957, the American Dairy Science Association suggested the following tolerances as a guide in determining accuracy:

(1) Butterfat tests on individual cows should be within 0.2 (+ or −) of the fat test obtained by conventional sampling methods.

(2) Daily milk weights, as compared to an accurate scale, should be within 3%, or one-half pound, whichever is greater, and the error should be random, so it will cancel with repeated weighings.

(3) Physical and mechanical limitations will result in occasional errors. However, 90% of the tests and weights should fall within the limits given here.

This should help manufacturers in developing devices for this purpose, and it places a definite responsibility on those supervising DHIA testing in each state and county, to determine which systems are satisfactory. Systems that differ from the conventional method for taking samples and weighing milk should be checked carefully. Methods for checking accuracy on weights and tests should be carefully designed to give results that can be measured in accordance with the tolerances listed for individual cows. Group averages are inadequate when determining the accuracy of different systems for weighing and sampling milk.

In any system, all precautions should be followed to assure accurate and authentic records. An error on test day may be magnified many times. Demonstrations of the different ways of weighing and sampling milk from individual cows, for our dairy testers, as well as for DHIA members, can be very helpful in pointing out the precautions that need to be taken and for recognizing the systems that are inadequate.

What has formerly been a relatively simple, but always an extremely important part of production testing, is developing into a more complex problem, with our new systems of weighing and sampling milk from individual cows. This should be a challenge to move forward with new developments in production testing, in order that this important tool for herd management can keep pace with other improvements in dairy farming.

Ruminology—An Interdisciplinary Science[1]

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Developments in theoretical science often influence human activities in some way. More effective, practical mastery of the environment, brought about by technical innovations made possible by such developments, may produce striking transformations in modes of social living. Since ruminant animals play an important part in the economic and social life of many of the peoples of the world, it is evident that increased understanding of the interactions between ruminant host and its microflora and microfauna would be valuable for many reasons. Possible practical results of such investigations which immediately suggest themselves are, for example, better utilization of cheap, readily available (synthetic) feedstuffs, increased meat, milk, and wool production; and to each of these would accrue economic and consequent social advantages, particularly in overpopulated, underdeveloped areas of the world.

In addition to mere practical gains, research in this area will yield much needed information concerning symbiotic, synergistic, and other interaction phenomena on the cellular level. In view of the many basic problems of the rumen, such as those of microbial ecology, the role of microorganisms in degrading biochemically inert (to the host) or refractive polymers, the relation of rumen digestive processes to over-all ruminant physiology, the factors stabilizing the rumen (so that it can be considered, in fact, a liquid organ), the role of the rumen in metabolic diseases of ruminants—as, for example, bloat, lactation tetany, and ketosis, there has arisen a need for developing and refining a body of valid techniques that will aid in establishing fundamental knowledge upon which a comprehensive theory of rumen function can be constructed. Ideally, such a theory ought so to organize and unify knowledge in this field that as-yet-unrealized relationships are made apparent. In order to characterize this area of scientific inquiry, the word "ruminology" (L. pl., *rumina*, rumens, plus G. or L., *logia*, a science, hence the science of rumens) was coined *(12)*, and it is defined as that branch of biology which deals with the study of the rumen in any of its manifestations. Investigations undertaken on

[1] Scientific article No. A-622, Contribution No. 2804 of the Maryland Agricultural Experiment Station.

problems such as those cited above require techniques borrowed from microbiology, chemistry, protozoology, physiology, and animal husbandry. In addition to modifying existing techniques of various established disciplines, there must be developed new and original methods relevant to ruminology, and distinctly characteristic of it as a science rather than as a "practical art."

The single, and perhaps most important, feature of the rumen is its microflora and microfauna. Early work, typical of its time, was devoted to microscopical and pure culture investigations of the rumen liquor of cows, sheep, and goats. In the main, this work dealt with attempted characterizations of bacteria capable of hydrolyzing cellulose and the general problem of cellulose utilization by ruminants. A singular feature of the rumen microflora is that many of the microorganisms found therein have not been described as existing elsewhere in nature, even in cellulose-rich microenvironments. As early as 1882, observations were made on incubated rumen contents and, later, interesting studies of bacteria attached to plant fragments in the rumen were carried out. Investigations of this sort were brought to their ultimate development in an extended series of papers by Baker and his colleagues *(1-4)*. It is now clear that such microscopical studies and associated techniqes (staining, counting), while yielding interesting and sometimes suggestive data, are not sufficient tools for characterizing the activities of authentic rumen microorganisms.

Cultural work of value was begun around the 1930's and foundations were laid for procedures still employed at the present time. Hungate, in a brilliant series of investigations *(15, 16, 17)*, first isolated, maintained, and characterized morphologically and physiologically, pure cultures of cellulolytic rumen bacteria, and he developed the specialized techniques required, to the point where reproducible results can be obtained easily. Since his work, the ecological approach of Beijerinck has yielded such fruitful results *(8, 9, 11)* that it is now possible to construct a fairly extensive table *(33)* charting the major characteristics and probable function of 15 species of rumen bacteria.

Some recent attempts to study rumen microorganisms *interacting*, either as resting cells or in so-called "artificial rumens", have been promising *(14, 32, 31)*. It is perhaps trite to state that analysis of events taking place in an environment involving more than one species of organism may become exceedingly difficult when this must be done on the microbial level, particularly if one allows growth to occur. At present, it seems wise to study mixed suspensions of rumen microorganisms as tissues, and to consider the over-all reactions as resultants of enzymatic competition among them as they are prepared in the resting state; thus, one can bring to bear on them all of the usual techniques used for the study of tissue metabolism *(15)*.

Excellent accounts of rumen protozoa have been published *(6, 21)*. Difficulties in freeing species of protozoa from bacteria, and from each other, and the observation *(5)* that defaunated ruminants continue to thrive, have had a dampening effect on investigations dealing with ruminal functions of these interesting organisms. Recently *(18, 23, 24)*, interest has been revived by some elegant experimental work, the net result suggesting that rumen protozoa may be important to the host for a variety of reasons, among these: *(a)* that they may be cellulolytic; *(b)* they may be of nutritional value to the host; *(c)* they may aid in the attack on starch and other carbohydrates; and *(d)* they store starch, which may serve as a reserve substance in the rumen.

In concluding this brief survey of some features of microbiology as included in ruminology, it might be wise to recall the observation of Oxford *(25)*, that by fermenting the most resistant fodder constituent (cellulose) at the beginning, rather than at the end, of its digestive tract, the ruminant has necessarily acquired a large number of microbiological problems that would prove exceedingly difficult to a fermentation chemist. It is not possible (though some would consider it desirable) to maintain in the rumen a mixed culture devoted almost exclusively to the desired end only; namely, cellulose fermentation to acetic, propionic, and butyric acids with B-vitamin production.

The interaction of ruminant host and its microflora and microfauna is well-illustrated in the relationships between digestive processes and over-all physiology. It has long been thought that ruminants might derive protein from nonprotein nitrogen through the rumen microorganisms, but it has been only recently that the protein-sparing action of urea was recognized *(30)*. There is no doubt that under optimum conditions, governed in part by the age of the animal, type of diet, and various factors influencing the microflora, urea can be converted to host protein. A great deal of work has yet to be done on the possibility of feeding ruminants synthetic materials and various by-products for which there is no use at present.

Phillipson and coworkers *(29)* were pioneers in the investigation of the volatile fatty acids of the rumen. They showed that cellulose, starch, and a variety of carbohydrates were fermented to those acids, which, of necessity, serve as the chief energy sources of the host. The principal fatty acids of the rumen later were identified as acetic, propionic, and butyric *(13)*. Originally, it was thought that they were merely absorbed through the rumen, but recent work *(26, 27)* has shown that the rumen epithelium actively metabolizes these acids.

Digestive enzymes are not secreted into the

rumen, nor are they present in the saliva *(10)*. There is no organized body of knowledge dealing with protein digestion in the ruminant, as contrasted to the nonruminant animal, and this can be explained partly on the basis of preoccupation with cellulose digestion and interest in synthesis of protein from nonprotein sources. A large gap exists between the data obtained from feeding trials and those obtained from in vitro experiments, and it remains for the ruminologist to integrate these data into something more meaningful.

Vitamin synthesis in the rumen has been known for some time *(20)* and it is evident that the mature ruminant is independent of exogenous supplies of B-vitamins. Present work includes defining the conditions and kinds of organisms involved in vitamin synthesis, as well as identifying unknown factors. It is known that trace minerals, particularly cobalt, copper, molybdenum, calcium, and phosphorus are important in ruminant nutrition *(22)*. Some trace metal requirements undoubtedly reflect those of the rumen microorganisms. In a few instances (as, for example, cyanocobalamin formation), synthesis by rumen microorganisms and trace metal requirements overlap. Techniques required for investigations in this field must be developed further and, in fact, the location of areas deficient in certain trace metals and the study of microenvironments involved in vitamin synthesis in a large area have been termed "biochemical geography" *(19)*.

In the case of certain diseases of ruminants, as bloat and ketosis, the interaction between microflora, feedstuffs, and host is very evident. In the former disease, gas formation, fatty acid production, viscosity increase (microbial capsule formation?), and surface tension phenomena, among other things, seem to be involved *(28)*. In the latter disease, fatty acid production and disturbances in the pituitary-adrenal system are involved *(31)*. In these situations the traditional boundaries of veterinary medicine, biochemistry, physiology, and animal husbandry are blurred out. Work in these cases can not be done successfully except by research teams composed of specialists or, as suggested here, by ruminologists. Further, anyone desiring to make a contribution to our knowledge of these diseases must possess some information from each of the above-mentioned fields, yet not necessarily be a specialist in any one of them. Various other disturbances, such as lack of synthesis in the rumen, scouring, and low butter fat also are in this category. A pressing need is manifest for meaningful and intelligent research on: the effects of antibiotic feeding; the physiological and ruminal differences between young and adult animals; the conditions of feeding considered normal for ruminants; how to circumvent, or at least give direction to, expensive, time-consuming feeding trials, by development of valid in vitro techniques *(7)*, and the introduction and effects of using synthetic feedstuffs.

Many of the observations concerning the rumen have been made by investigators working in several independent disciplines. A number of papers have been published in which no attempt was made to relate to the intact animal the data obtained. Thus, one discovers that some microbiologists are content to consider the rumen as nothing but a fertile field for describing organisms with new or unusual biochemical abilities; that some animal husbandrymen are content to consider the rumen as nothing but a fermentation vat, for producing precursors for milk synthesis or for transforming forages to flesh; that some veterinarians look upon the rumen as an ideal spot for administering antibiotics and a host of agents, with miraculous, if vague, properties, and that some biochemists find the rumen an ideal location for the isolation of new enzymes. In this cursory exploration, stress has been placed on relationships important to the over-all field rather than to any one part, and it is hoped that specialists will see the value of viewing their problems in relation to the whole of ruminology.

In any event, it is certain that continued research in ruminology will provide fruitful benefits for related branches of biology, the practitioners of which may be surprised at thus receiving new points of view from a hitherto-unexpected quarter.

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OUR ASSOCIATION

ACTIVITIES OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

C. F. Huffman
Past President

The activities of our Association are far greater than most of us realize. As a matter of fact, it took a term as President for the writer to realize the scope of the activities of this great organization of more than 2,200 members. A sketch of some of the happenings during the past year will give you a greater appreciation of our organization.

The high honor of serving you as President is deeply appreciated, but the many chores can also be burdensome. The look of satisfaction on the face of Ira Gould when he turned the job over to the writer in 1956, can now be appreciated.

The President has many duties. The appointment of most of the committees of the Association is a Herculean task. It is necessary to take into consideration not only the qualifications of those appointed, but also to take into consideration the representation of various sections of our Association and the regions of the country.

The President also has the responsibility of keeping the Executive Board informed of the business of the Association. In case of actual need for Board action, it is necessary for the President to either poll or convene the Board. In practice, however, the only meetings of the Executive Board usually occur just before and during the Annual Meeting. There are occasions when there is a real need for convening the Board between Annual Meetings, but our limited budget will not permit it.

It is the duty of the President to send a welcoming letter to all new members. Letters of condolence are sent to the next of kin of our deceased members. The new Administrative Handbook prepared by Secretary Judkins lists 15 different duties of the President.

Recently, additional duties have been assigned to the Vice-President. He now has the responsibility of assembling and preparing committee reports for use at the business sessions of the Annual Meeting.

The greatest center of activity of our Association is confined to the office of the Secretary-Treasurer. It is a beehive of action. H. F. Judkins is a part-time officer in salary only. He sends out notices of dues payable and other notices, collects dues, and handles advertising. As a matter of fact, a list of his duties covers four pages in the Handbook. During the past year H. F. Judkins was my good right arm. He not only did more than was expected of him at every turn but was always most congenial. It has been a pleasure to work with him.

One of the most important activities of the Association is the publication of the *Journal of Dairy Science*. The marked improvement in the Journal during the past few years has been due largely to the untiring efforts of

The Board of Directors in action at the 52nd Annual Meeting, Oklahoma State University, June 26-29. Sitting: E. H. Parfitt, G. Hyatt, Sec.-Treas. H. F. Judkins, Maxine Frank and Patsy Billy, secretaries, Pres. C. F. Huffman, Vice-Pres. D. V. Josephson, R. E. Hodgson, I. A. Gould. Standing: G. M. Werner, E. L. Fouts, Editor E. O. Herreid, I. W. Rupel.

P. H. Tracy, our former Editor. The burden of work became so great that Editor Tracy resigned December 31, 1956. The Executive Board appointed a special committee consisting of W. V. Price, W. M. Roberts, and myself to find a new home for the Journal and to work with the Journal Management Committee to select a new Editor. W. V. Price received bids from several publishing houses on costs for handling routine editorial services. The committee recommended the selection of The Garrard Press, which was approved by the Executive Board. R. J. Dandeneau is now handling these services for The Garrard Press. The Garrard Press also took over the handling of our Journal subscriptions in order to ease the pressure in the Secretary-Treasurer's Office.

Although this arrangement has greatly lessened the work of the Editor, he, with the help of the Editorial Board, still has the responsibility of approving all papers, manuscripts, and abstracts published in the Journal.

The special committee, with the cooperation of the Journal Management Committee, persuaded E. O. Herreid, an associate of P. H. Tracy, to accept the Editorship. The Executive Board was very pleased with this selection. Editor Herreid has a lot of splendid ideas that will improve our Journal still further.

Finances for our Association are derived from dues, subscriptions to the Journal, sale of reprints, and the sale of advertisements in the Journal. The profit from the sale of reprints passes on to the authors' institutions some of the cost of financing the American Dairy Science Association. As a matter of fact, the sale of advertisements and reprints aids materially in keeping down membership dues.

A large amount of the work of our Association is carried out by numerous committees. The names of the committee members appear each year in the January Journal. In addition to the Association Committees, there are numerous other committees in the Manufacturing, Production, and Extension Sections. These sectional committees aid in solving many problems in their respective fields. All committees contribute very markedly to the progress of our Association, but because of limited space, only a few will be mentioned in this communication.

In order to do a more effective job of promoting the welfare of the dairy industry, it is necessary for us to increase our membership and subscription lists. Although our membership has increased appreciably during the past few years, the rate of increase should be stepped up. President Josephson has appointed J. B. Frye as Chairman of the Membership Committee for this year. It is difficult for our national and state chairmen to get this important job done without the active cooperation of all of our members. Personal contact is still the best method for getting new members. Please, let's give the membership committee a helping hand.

We are proud of the development and success of the Student Affiliate Committee. We have 37 active chapters at the present time. These active chapters, working at the grass-roots level, contribute to our Association by stimulating an interest in the welfare of our industry. This is reflected in an increase in the regular membership of the student affiliates who have finished their college work. The Student Affiliate Committee, with W. L. Slatter as Chairman, deserves a lot of credit for the development of leadership for the future of our industry.

We also have Eastern, Western, and Southern Divisions of the American Dairy Science Association. These divisions give the workers in the respective areas an opportunity to get together and have their own programs relatively close to home. The Association-Division Coordination Committee is composed of the chairmen of the respective Divisions, with H. F. Judkins as an ex-officio member.

There should be a closer tie-up of the Divisions with the parent organization. A start has been made in this direction by the attendance of American Dairy Science Association representatives at the Divisional Meetings. During the past year, I. W. Rupel, Executive Board Member, represented us at the meeting of the Western Division. Dr. Rupel was given the opportunity to discuss our Association at this meeting and as a result he suggested that abstracts of the scientific papers presented at these meetings be published in the Journal. This was done. Our congenial Secretary, H. F. Judkins, attended the Eastern Divisional Meeting. It was my privilege to attend the meeting of the Southern Division. I was greatly impressed with the quality of the scientific reports and with the amount of time devoted to helping the parent association. Strong Divisions do us a lot of good.

Louisiana has a State Division which the Executive Board recently authorized on an experimental basis.

The success of the Annual Meeting depends, to no small degree, on the quality of the program that will be presented. The selection of the program is the responsibility of the Association's over-all Program Committee, headed by N. P. Ralston, and the Chairmen of the Manufacturing, Production, and Extension Sections. The host institution also has a representative on this committee. The Association committee, together with members of the Sectional program committees and the current President, meet in Chicago the latter part of November to finalize the program. If any of you have any criticisms or suggestions for

improving the program, they should be sent to the Chairman of the Program Committee prior to the November meeting.

The American Dairy Science Association, because of the varied talents of its members, is interested in rendering service to other dairy organizations. The Personnel Manual Preparations Committee is in the process of preparing a manual to be used for training workers in dairy plants. The Public Health Liaison Committee works closely with the United States Public Health Service and other public health agencies to bring about a more active program of collaboration between your Association and these public health agencies.

Some 14 dairy organizations have representation on the Dairywide Coordination Committee on Nutrition. H. F. Judkins is Chairman of this committee this year. Our Association has three representatives on this committee. This committee reviews all scientific data on the nutritive value of milk and milk products and suggests research projects needed to fill the gaps. It also makes recommendations for the use of research findings in public relations, advertising, and educational work.

Our Association is working with the Subcommittee on Education and Training of the Dairy Industry Committee (D.I.C.). We also have a representative on the 3-A Standards Subcommittee of D.I.C. H. F. Judkins handled the American Dairy Science Association booth at the Dairy Industries Exposition held in Atlantic City last fall. The President had the honor of taking part in the opening ceremonies of "Frontiers of Dairy Science Day" at this meeting. C. G. Bradt represented the Association at the dedication of the Plum Island Animal Disease Laboratory. W. E. Petersen was our representative at the annual meeting of the National Artificial Breeders Association.

Our Association also has representatives on the National Research Council, Committee on Inter-Society Cooperation, Dairy Remembrance Fund, American Association for the Advancement of Science, American Institute of Biological Sciences, and the Ralston-Purina Research Fellowship Committee.

The Annual Meeting held in Stillwater, Oklahoma, in June of this year marked the first time that our Association had visited the Southwest. As is the rule, this meeting was the climax of our year's activity. Our gracious hosts anticipated our most trivial needs. The friendly atmosphere prevailing in Stillwater was greatly appreciated and will be long remembered by those of us in attendance.

The Past Presidents' Dinner was a great success. This dinner gave us the opportunity to obtain valuable suggestions from those who have had many years of experience. These suggestions may help the present officers solve some of their important problems.

The barbecue held in the stadium was a gala occasion. The food was exceptionally tasty and the Indian Dance by real Oklahoma Indians gave all of us a thrill. The President was thrilled when he was called to the field to meet the Indians. He was greatly relieved, however, when they decided that his scalp was not worth taking. They kindly made him an Honorary Chief and then gave him a lesson in an Indian dance.

The Awards Program held on the last evening was an exciting one. President Josephson and Secretary Judkins were awarded the title of Honorary Colonels of Oklahoma. The three Student Affiliate display contest winners received citations.

Very few Presidents have had as much satisfaction from the Awards Program. Dr. G. Malcolm Trout, a long-time associate of the President, received the Teacher's Award; J. T. Reid, a former student, now on the staff of Cornell University, received the Borden Award in Dairy Production, and the President had the honor of presenting Earl Weaver, an old friend and associate at Michigan State University for many years, the Honor Award.

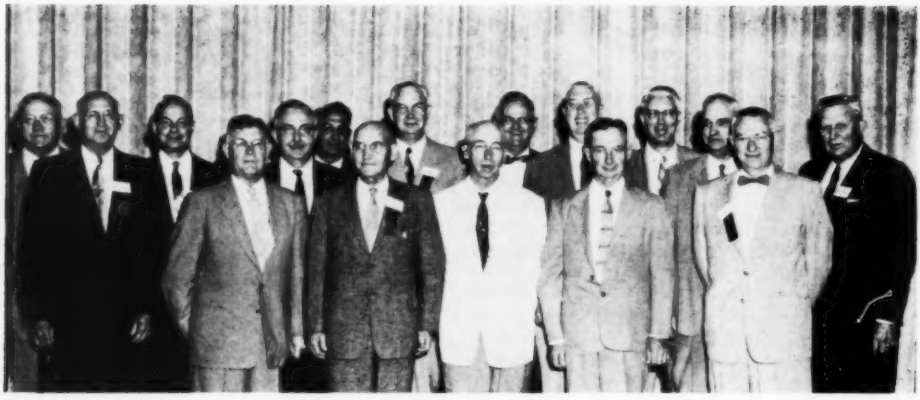
This opportunity is taken to express deep appreciation to all the committees, officers, and members of our Association who were so helpful during my year as President.

PAST PRESIDENTS RIDE AGAIN

G. Malcolm Trout
Historian

"Don't drive an old fire-horse to a fire unless you want him to run," must have been in Vice-President Josephson's mind when he invited all the Past Presidents of the American Dairy Science Association to a special dinner on the eve of the 52nd Annual Meeting, Stillwater, Oklahoma, Tuesday, June 18. Not only did 17 of the 28 living Past Presidents meet with the members of the Executive Board at the dinner, but they kept up a two-hour running debate on past, present, and future policies of the Association. When the debate was at its peak, President Huffman, sensing an endless discussion, snuffed out the fire suddenly, with the gavel—and to everyone's satisfaction.

The "old boys" ran true to form. The spark which they had as one-time ranking officers soon kindled into flame. Seemingly, all at once, the



Past Presidents at Oklahoma, 1957

Left to right: J. A. Nelson, G. M. Trout, H. B. Henderson, E. Weaver, W. E. Petersen, L. A. Moore, C. L. Roadhouse, H. C. Jackson, R. B. Becker, H. F. Judkins, H. P. Davis, J. B. Fitch, I. A. Gould, A. C. Ragsdale, W. V. Price, and H. W. Gregory.

atmosphere was charged with ideas—some good, others not so good. Following introductions, President Huffman needled the Past Presidents by announcing their subject, "Brain-Storming on the Association." His mental arithmetic revealed a total of 680 years of dairy experience among those present. Professor J. B. Fitch, Minnesota, alone had 47 years of dairy experience to his credit. Thus, Huffman urged exchange of ideas emanating from their many and varied experiences. Some of the suggested topics listed for discussion were:

(1) What more can be done to increase and keep new membershp?

(2) What to do about public relations?

(3) Is our Journal and program approach too technical?

(4) Is our Administrative and Board structure adequate to serve A.D.S.A. business and objectives effectively?

(5) The trials and tribulations of the Editor.

The discussion led off on "How to Increase Membership." Secretary Judkins was pleased to announce that as of then the Association had 2,196 members, exclusive of life, charter, and honorary members. Circulation of the Journal was around 4,300. Editor Herreid reported that although the American Dairy Science Association was increasing the fourth fastest among scientific societies, a faster increase was needed. Despite these favorable conditions, anxiety was felt over a possible slowdown of further increase in membership. Discussion brought forth many problems and situations which needed to be resolved. As expected, rising costs was one of the first items discussed. Other topics commanding attention were as follows:

(a) Many qualified industry personnel should be members of the Association; *(b)* possibility of group subscriptions to the Journal for dairy undergraduates; *(c)* many members of regulatory agencies might well be affiliated with A.D.S.A.; *(d)* little personal solicitation for membership by present members; *(e)* laboratory personnel a potential source of new members; *(f)* kinds of membership afforded by the Association; *(g)* effect of type of Journal articles on membership appeal; *(h)* classification of articles so as to reach the practical dairyman; *(i)* "one article put to use, worthy of price of Journal"—Dr. R. B. Becker; *(j)* student affiliates as potential members; and *(k)* increasing membership in commercial field by: 1) programs of interest; 2) solicitation of ideas; 3) invitation of leaders; 4) citation awards for distinguished service, and 5) special-interest meetings tied in with the annual A.D.S.A. meeting.

Dr. C. L. Roadhouse, professor emeritus, Davis, California, and President of the Association in 1935, reviewed the effective methods of the past in securing membership, stating that personal solicitation was most effective and that the potential of conscientious key members in each state in securing new members must not be overlooked.

Dr. Earl Weaver, Coordinator of Point 4 Program, Colombia, South America, and President of the A.D.S.A. in 1939, believed that membership increase among commercial men could be had, in part, through encouragement of responsibility in the Association. Editor Herreid replied that difficulty was encountered in getting commercial men to accept responsibility in the Association because they were so

busy elsewhere. Dr. E. H. Parfitt, former Purdue professor, and now Executive Director, American Evaporated Milk Association and director, A.D.S.A., expressed the commercial view that the Association too frequently was considered a college group. He was convinced that in order to interest commercial men in the Association, a public relations program should be instituted, and that an attempt be made to find out what industry wants for a program. He cited the success of the annual evaporated milk dinner program, held on the eve of the annual A.D.S.A. meeting, as an effective means of enticing industry to the Association meeting.

Vice-President Josephson announced the appointment of a Policy Committee, to advise or give the Executive Board a blueprint of the American Dairy Science Association. The committee consisted of Floyd Arnold, J. H. Erb, I. A. Gould, Ralph Hodgson, with Walter V. Price as chairman.

The new committee was urged to investigate the various ideas proposed, as well as the advisability of holding associative meetings concurrently, before or after the annual Association meeting. Dr. Ralph Hodgson, USDA, expressed the general feeling that the newly appointed committee should always keep uppermost in mind the objectives of the Association and what it is expected to do.

Upon motion by Professor H. P. Davis, Nebraska, President 1943, and seconded by Trout, a unanimous vote of confidence was given the present Executive Board for its excellent management of the affairs of the Association.

The Executive Board, consisting of Messrs. Huffman, Josephson, Judkins, Herreid, Hyatt, Rupel, Werner, Parfitt, Fouts, Hodgson, Turk, Jack, and Herman seemed to have been pleased by the open, free discussion by the old firehorses. In fact, the Past Presidents felt they had a good run, and secretly hoped that the Past Presidents Dinner, inaugurated under President Gould's regime in 1956, would become an annual affair.

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JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

BOOK REVIEWS

470. Patent Developments in the Cheese Industry, 1900 Through 1951. D. M. IRVINE AND W. V. PRICE. Dept. Dairy Industry, Univ. of Wisconsin, Madison. 80 pp. 25¢. 1952.

For anyone in the cheese industry who is doing research and is anticipating applying for a patent, here is a publication that is extremely valuable. Although this pamphlet has a publication date of 1952, and was originally published for limited circulation, it is now available for general distribution. The authors point out that approximately 600 patents relating to the cheese industry have been granted since 1900. They have classified these patents as they relate to type of cheese, equipment, merchandising aids, authors, and data.

To illustrate the completeness of the work, the following outline shows the manner of classification of the patents:

I. Cheese manufacturing: *(a)* Blue; *(b)* Cheddar; *(c)* Cottage and Cream, *(d)* Swiss. II. Equipment: *(a)* Curd mills; *(b)* hoops, molds, followers, and bandages; *(c)* for cheese manufacturing; *(d)* presses, *(e)* vats. III. Curing and curing supplies: *(a)* boxes; *(b)* coating and packaging; *(c)* dehydration; *(d)* handlers and holders; *(e)* mold prevention; *(f)* paraffining; *(g)* preserving, *(h)* ripening. IV. Process Cheese: *(a)* making; *(b)* equipment; *(c)* emulsifying agents, *(d)* cheese foods. V. Merchandising acids: *(a)* covers; *(b)* cutters. VI. Miscellaneous patents. VII. Foreign patents. VIII. Author index. IX. Subject index.

S. L. Tuckey

471. Breeding and Improvement of Farm Animals. 5th ed. V. A. RICE, F. N. ANDREWS, E. J. WARWICK, AND J. E. LEGATES. McGraw-Hill, Inc., New York, N. Y. 537 pages. $8.50. 1957.

The fifth edition of "Breeding and Improvement of Farm Animals" will be welcomed by the many persons who have used and appreciated the former editions. The senior author again has prevailed on the assistance of Dr. F. N. Andrews in the reproduction field, and Dr. E. J. Warwick in the field of animal breeding. In addition, the services of Dr. J. E. Legates have been secured, to handle the treatment of variation, population genetics, and dairy cattle selection. The combined efforts of these authors have produced a comprehensive text.

The material in the fifth edition is presented in logical, easily understandable manner. The introductory chapter is followed by three sections which are entitled, I. Physiology of Reproduction, II. Mechanisms of Heredity and Variation, and III. Breeding Systems and Selection.

In the first section, the anatomy and physiology of the male and female reproductive systems are described. Other chapters in this section give a description of the physiology of spermatozoa and ova; pregnancy, parturition, and lactation; lowered fertility and sterility; breeding management practices, and artificial insemination.

In the second section, in addition to chapters on the qualitative and quantitative aspects of heredity, the chapters on variation and population genetics add greatly to the value of this text. The subjects of sex determination, sexual differentiation, and lethal effects of genes complete this section.

The third section gives an outline and discussion of the systems of breeding and the general principles of selection. In this section, also, a chapter each is devoted to selecting dairy cattle, beef cattle, sheep, and swine.

The text is supplemented with numerous diagrams and tables. The scope of references which is found at the end of most of the chapters will be of value for further reading on the subjects. In addition, a comprehensive list of the Purebred Registry Associations of the United States is given in the Appendix.

R. G. Cragle

BUTTER

472. Automatic slicer for bars of butter or the like. F. BRODSKY. U. S. Patent 2,798,293. 1 claim. July 9, 1957. Offic. Gaz. U. S. Pat. Office, 720, 2:249. 1957.

A device for slicing butter in block form into individual parts separated by a paper strip.

R. Whitaker

CHEESE

473. A simplified short-time method for making Cheddar cheese from pasteurized milk. H. E. Walter, A. M. Sadler, J. P. Malkames, and C. D. Mitchell, Eastern Utilization Research Branch, Beltsville, Md. S. Dairy Prod. J., **60**, 3:22. 1956.

A short-time method of making Cheddar cheese from pasteurized milk, which uses conventional equipment, is described. The method has been developed on a pilot-plant scale and is ready for trial under commercial conditions. Two starters are used: the conventional lactic starter and a heat- and salt-tolerant culture of *Streptococcus durans*. Starters and rennet are added to the milk at 88° F. and the cut curd is cooked to 100° F. The salted curd is hooped at about 110° F. in a small volume of salted whey or salted water. The method requires only three hours until the curd is pressed. An excellent flavor develops upon ripening for three months at 55° F. The cheese is considered to be superior in body and texture to that made by present methods, being practically free of mechanical holes. F. W. Bennett

474. Present-day problems in the manufacture of cottage cheese. N. C. Angevine, Meyer-Blanke Co., St. Louis, Mo. S. Dairy Prod. J., **60**, **4**:36. 1956.

Trends in the manufacture of cottage cheese during the last few years have been greatly toward mechanization, standardization of procedures, improved sanitation, and increased shelf-life. Laboratory control is being given more attention. Details of all parts of the manufacturing procedure and control are reviewed.
F. W. Bennett

475. Method of manufacturing cheese. H. E. Walter, A. M. Sadler, J. P. Malkames, and C. D. Mitchell. U. S. Patent 2,796,351. 6 claims. June 18, 1957. Offic. Gaz. U. S. Pat. Office, **719**, 3:576. 1957.

Milk is inoculated with a starter of lactic acid streptococci which do not produce acid in salt concentrations of 4% and at temperatures above 105° F., and a culture of *Streptococcus durans*. After ripening, rennet is added, the coagulated milk cut, and the curd cooked to from 95 to 105° and drained. The curd is heated to from 115 to 118° with a small amount of whey, salted to 4% salt, pressed, and curded. R. Whitaker

CONDENSED AND DRIED MILKS; BY-PRODUCTS

476. The manufacture of quality buttermilk. N. C. Angevine, Meyer-Blanke Co., St. Louis, Mo. S. Dairy Prod. J., **60**, 5:120. 1956.

Cultured buttermilk is superior to the old-fashioned buttermilk of the past. It is the most profitable item a dairy sells, and it fits in with today's weight-reducing trend. Uniformity of product is essential. Important steps in the making procedure include fresh, clean-flavored milk, preferably 1% fat and homogenized; heating to from 180 to 185° F. for 30 minutes and cooling to from 70 to 72° F.; use of clean, active, smooth-bodied cultures with aroma; special stainless vats with large, slow-speed agitators and adequate facilities for cooling; breaking at pH 4.4, or at 0.85 to 0.90% acidity; prompt and rapid cooling and storage at 40° F. or lower. The product may be made more attractive by the addition of butter flakes or granules.
F. W. Bennett

DAIRY CHEMISTRY

477. Improved method for the preparation of crystalline β-lactoglobulin and α-lactalbumin from cow's milk. R. Aschaffenburg and J. Drewry, Chem. Dept., N.I.R.D., Univ. of Reading. Biochem. J., **65**, 2:273. 1957.

A simple scheme is described for separating β-lactoglobulin and α-lactalbumin in the so-called "lactalbumin" fraction of the milk proteins. The lactalbumin fraction is prepared by salting out the casein and globulins with Na_2SO_4 (20 g/100 ml.). The salt filtrate is adjusted to pH 2; whereupon, all of the protein constituents, with the exception of β-lactoglobulin, precipitate. This scheme permits the simultaneous or independent working up of the β-lactoglobulin and α-lactalbumin. The method is applicable to bulk milk and to the milk of individual cows containing β_1- or β_2-lactoglobulin, either singly or in combination. β_2-lactoglobulin crystallizes more readily if seeded with the orthorombic form of crystals, rather than with the monoclinic form. R. Jenness

478. Preparation and amino acid composition of enzymically dephosphorylated casein. T. A. Sundararajan and P. S. Sarma, Univ. Biochem. Lab., Madras, India. Biochem. J., **65**, 2:261. 1957.

A phosphoprotein phosphatase free from proteolytic activity was prepared from ox spleen and employed to dephosphorylate casein. The dephosphorylation was performed at pH 6 and the product precipitated as it was formed, since it is insoluble at this pH. During the dephosphorylation process small amounts of acid-soluble nitrogen, amounting to about 2% of the total nitrogen of the casein, were liberated. Upon acid hydrolysis and paper chromatography of the acid-soluble peptide fraction, a number of amino acid constituents, including glutamic acid, isoleucine, and serine, were revealed. The dephosphorylated casein contained 15.55% nitrogen and 0.04% phosphorus. Its amino acid composition did not differ markedly from that of the original casein. It was concluded that the enzymatic dephosphorylation of casein leaves the molecule relatively intact, except for the cleavage of phosphorus-containing peptides. R. Jenness

479. Biosynthesis of fatty acids in cell-free preparations. 4. Synthesis of fatty acids from acetate by a partially purified enzyme system from rabbit mammary gland. P. HELE, G.POPJÁK, AND M. LAURYSSENS, Med. Research Council, Experimental Radiopathology Research Unit, Hammersmith Hospital, London, W.12. Biochem. J., **65**, 2:348. 1957.

A partially purified enzyme system was prepared from lactating rabbit mammary gland. This catalyzed the synthesis of even-numbered-chain fatty acids from C4 to C18, with the shorter-chain fatty acids predominating. Essential reactants for the synthesis were acetate, co-enzyme A, ATP, and stoichiometric amounts of reduced DPN. The intermediates in the reaction were studied by paper chromatography as their hydroxamates. The data support the hypothesis that fatty acid synthesis in these preparations occurs by a stepwise condensation of C_2 units, through the reversal of the β-oxidation of even-numbered-chains. R. Jenness

480. Glycerol metabolism by spermatozoa. T. MANN AND I. G. WHITE, A.R.C. Unit of Reproductive Physiol. and Biochem., Univ. of Cambridge. Biochem. J., **65**, 4:634. 1957.

Ram spermatozoa, separated from seminal plasma by centrifuging and washing, metabolized glycerol aerobically to lactic acid. The presence of phosphate increased the yield of lactic acid by inhibiting the oxidation of the lactic acid by spermatozoa. Dihydroxyacetone also was oxidized by spermatozoa as rapidly as glycerol, giving rise to lactic acid. Both α- and β-phosphoglycerol increased the respiration of spermatozoa because of the oxidation of glycerol set free by dephosphorylation. Glycerylphosphorylcholine, a natural constituent of ram semen, was neither dephosphorylated nor oxidized by spermatozoa. R. Jenness

481. Glycerylphosphorylcholine and phosphorylcholine in semen, and their relation to choline. R. M. C. DAWSON, Biochem. Dept., A.R.C. Inst. of An. Physiol., Babraham, Cambridge, T. MANN and I. G. WHITE, A.R.C. Unit, of Reproductive Physiol. and Biochem. Univ. of Cambridge. Biochem J., **65**, 4:627. 1957.

It was demonstrated by paper chromatography that glycerylphosphorylcholine and phosphorylcholine account for the bulk of the water-soluble bound choline and the acid-soluble, difficultly hydrolyzable phosphorus in the seminal plasma of various mammals. In man, the content of phosphorylcholine exceeded that of glycerylphosphorylcholine, but in the ram, bull, boar, stallion, goat, and rabbit, practically all of the bound choline was accounted for as glycerylphosphorylcholine. The presence of glycerylphosphorylcholine in ram serum was confirmed by isolation of 470 mg. of crystalline cadmium salt from 40 ml. of seminal plasma. Trace amounts of glycerylphosphorylethanolamine were detected in some samples of some seminal plasma, but phosphorylethanolamine was completely absent. Phosphorylcholine was readily hydrolyzed in semen after ejaculation, but glycerylphosphorylcholine was neither dephosphorylated nor changed in any other way by spermatozoa. R. Jenness

482. Variations in the solids-not-fat content of milk. Investigations into the nature of the solids-not-fat. Problems in the West Midlands. T. W. GRIFFITHS AND J. FEATHERSTONE, Agr. Advisory Service, Wolverhampton, England. J. Dairy Research, **24**: 201. 1957.

The milk from seven herds was examined over the period from September, 1954, to August, 1956, on an individual cow basis. Solids-not-fat and butterfat were determined monthly and crude protein and lactose every three months. For various herds at various times the solids-not-fat (SNF) varied on a yearly basis from 8.20 to 8.73% for an over-all mean of 8.43 $\pm$ 0.404%. The poorest milk was produced during the late winter months, and the best quality during May and June. The per cent of samples falling below 8.0% SNF ranged from 28.8% in April to 13.4% in June (70% below 8.5% SNF in March and April). The lactation trend of SNF was essentially the reverse of the yield trend. Changes of SNF were mainly the reflections of changes in crude protein. Advance in age of cow definitely caused a lowering of SNF (0.11% per lactation). Friesian cattle gave milk with SNF lower than milk from Ayrshires. Careful supervision of the feeding of the herds did not increase the SNF content of milk. It was concluded that the major portion of the problem is the genetically poor potentialities of the cows. J. D. Donker

483. Synthesis of lactose in vitro by mammary gland slices from lactating rat, guinea pigs, and sheep. W. G. DUNCOMBE, N.I.R.D., Univ. of Reading, England. J. Dairy Research, **24**: 171. 1957.

Radioactive glucose and pyruvate used together or singly (one radioisotope at a time) were incubated with washed slices of active mammary-gland slices in a Kreb's bicarbonate ringer solution for 3 hr. at 37° C. No radioactivity was incorporated into lactose when pyruvate was radioactive and the only substrate. Inactive glucose added to active pyruvate caused the lactose to become active, but not as active as when glucose was radioactive. There was an increase in the concentration of lactose from zero-time in all cases where glucose was included in substrate. J. D. Donker

DAIRY ENGINEERING

484. How far away is the push-button hardening room? E. T. HOLLAND, JR., Richmond Mfg. Corp., Englewood, N. Y. Ice Cream Field, **69**, 6:48. 1957.

The author states that "automatic warehousing involves selectively moving all variations of inventory in and out of a storage area without the use of labor."

It is concluded that a push-button hardening room is a matter of application of machinery now available, rather than new machine design.

Details, including pictures and illustrations, are given for one system adaptable to automatic hardening rooms, which will discharge as high as 160 gal. per min. at conveyor speeds of 4 ft. per min. This system insures first-in–first-out control. W. C. Cole

485. Floor-type conveyors and automatic stacking. E. F. JUHREND, Mojonnier Bros. Co., Chicago, Ill. S. Dairy Prod. J., **60**, 4:33. 1956.

Automatic case-stacking machines may be either bottom-up stackers or top-down stackers. Their use greatly reduces physical labor by the handler and increases efficiency three to five times. Products from two fillers operating on different products with different-sized containers may be handled by a single case-stacker.

Floor-type conveyors have both the carrying and the return chains imbedded in the floor. They allow free movement from one area to another, make possible cleaner appearance, and eliminate lifting of cases. Installation is more exacting than in the case of conventional conveyors. Painting is not required and no more maintenance is necessary than for conventional types. Lubrication should be provided on original installation. F. W. Bennett

486. The effect of elevated temperatures in processing dairy products. G. E. FOOTE, Chicago Stainless Equipment Co., Chicago, Ill. S. Dairy Prod. J., **60**, 6: 28. 1956.

The proper application of temperatures in excess of 219° F. to milk, as in the Roswell equipment, does not have a detrimental effect upon the product. The thermophilic and thermoduric bacteria which withstand conventional pasteurization are killed and the shelf-life of the products is increased. Richer flavor from the stabilization of food proteins, ice cream stabilizer savings up to 75% of the normal, lower curd tension, and drier ice cream are other advantages. The U. S. Public Health Service has accepted the Roswell pasteurizer for pasteurization at 200° F., with a holding time of 3 sec.

A high-temperature system must be capable of processing at temperatures up to 300° F., protect the physical properties of the food, be economical, have sharper instrumentation for product control, be designed to withstand product pressure up to 200 p.s.i. minimum, be capable of product flow velocities up to 18 ft/sec, be a continuous system, designed for cleaning-in-place, and have surfaces impervious to corrosion. F. W. Bennett

487. Confection-packaging apparatus. W. M. RICH, U. S. Patent 2,796,711. 7 claims. June 25, 1957. Offic. Gaz. U. S. Pat. Office, **719**, 4: 680. 1957.

A filler is described for placing flowable material, such as ice cream from the freezer, into cups and the cups into cartons.

R. Whitaker

488. Stamping apparatus. E. VANTLANDER (assignor to Am. Seal-Kap Corp.). U. S. Patent 2,796,827. 2 claims. June 25, 1957. Offic. Gaz. U. S. Pat. Office, **719**, 4:710. 1957.

A machine for stamping cover-all-type hoods and caps for glass milk bottles.

R. Whitaker

489. Defoamer for milk-filling machines. W. H. RIEDEL AND M. RIEDEL. U. S. Patent 2,796,894. 5 claims. June 25, 1957. Offic. Gaz. U. S. Pat. Office, **719**, 4:727. 1957.

A tubular device for reducing the foam on milk to a liquid as it enters the filling tube of a milk-bottle filler. R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

490. Frontiers of dairy science. C. F. HUFFMAN, A.D.S.A. S. Dairy Prod. J., **60**, 6: 50. 1956.

Frontiers for future progress in dairy science include more production per cow, greater and more intelligent use and more economical production of roughage, savings in labor and cost of housing cattle, improved health of cattle, bulk handling of milk, greater use of milk and its products, improvement of milk quality, increased utilization of solids-not-fat, more knowledge of human nutrition, new methods, and push-button equipment. F. W. Bennett

491. Educational objectives in the manufacturing phase of dairy science. H. B. HENDERSON, Univ. of Ga., Athens. S. Dairy Prod. J., **60**, 3: 18. 1956.

The drastic reduction in enrollment of dairy manufacturing students in very recent years has raised many questions on the most desirable educational objectives. There is a great need for dairy industry leaders and college professors to decide upon the best type of training for college students in this field. Salaries in this field, which are comparable with those in other vocations, and other conditions which would tend to keep the employees contented, would be one of the most effective recruitment tools. A personnel training program, such as is being designed by the American Dairy Science Association, should be a valuable aid. Information relative to the opportunities in the vocation and the training available should be brought to the attention of high-school students.

F. W. Bennett

492. Requisition forms for your distribution branches. J. R. ANDREWS, Midwest Dairy Products Corp., Ruston, La. S. Dairy Prod. J., **60**, 3: 48. 1946.

To facilitate the placing of orders for full truck-loads of frozen dairy products of assorted kinds, the use of a conversion factor for space for each item is recommended. The factor is obtained by dividing the volume of a package of each item into the total volume of the load space, and then dividing this number into the load capacity in gallons for a standard bulk container. The number of packages of each item is multiplied by its respective factor and the sum of the results for all items is balanced with the number of gallons of standard bulk ice cream in a load. The requisition form lists all items available, with their respective conversion factors and spaces for entering the number of units ordered, and the "space gallons" required for each item ordered. F. W. Bennett

493. Route sales in Austria. H. IWAN. S. Dairy Prod. J., **60**, 4:250. 1956.

Ten dairies owned by dairy companies sell milk, cheese, butter, eggs, and other dairy products in Vienna at a uniform price established by the state. The price of milk is 10¢ per qt. and it is sold in qt., pt., and ½-pt. bottles, and in large bulk quantities out of 25-qt. cans. There are no paper containers, but there is home delivery. Wages are about $75 per month. The trucks, mostly horse-drawn, are loaded from a conveyor which runs down the center of a yard. Delivery begins about 11:30 P.M. and a second load is delivered early in the morning. Keys for the stores served are provided for routemen who enter the stores, deliver milk, and pick up money for the previous day's delivery. Kraft cheese is sold through nearly every store in Austria. F. W. Bennett

494. The competitive race for qualified manpower. C. BROWN, JR., Knowlton's Creamery, San Antonio, Tex. S. Dairy Prod. J., **60**, 6: 22. 1956.

In the competitive race for manpower, higher starting salaries, recognition by top management officials, guidance toward a certain goal, recognition in dairy group activities, announcement of openings to students in colleges and professional societies, and scholarships, are suggested to the dairy industry. Recommendations to college students and graduates include, recognition of responsibility to the industry and their colleges, more training in business and English; effort toward good personnel and public relations, and the gaining of some practical experience before graduation from college.
F. W. Bennett

GENETICS AND BREEDING

495. Genetic connection between body size, milk production, and efficiency in dairy cattle. L. MASON, A. ROBERTSON, AND B. GJELSTAD, Inst. Animal Genetics, Edinburg, Scotland. J. Dairy Research, **24**: 135. 1957.

Data from Red Danish cattle upon farms and in bull-progeny testing stations in Denmark were utilized to study the production and efficiency of production as related to size of animal. Size was measured as weight, heart girth, or height at withers. The heritability estimate of size and feed-conversion efficiency approximated 0.5. Milk yield was highly correlated positively with efficiency because, upon theoretical grounds, this was the result of any standardized feeding scheme. There was a slight positive correlation between yield and size. It was suggested that a useful efficiency index to select dairy animals by, would be yield divided by height of animal. J. D. Donker

HERD MANAGEMENT

496. Nonsiphoning drinking bowl for cattle. V. R. BERG, V. H. BOHL, AND C. S. MUELLER (assignors to Berg Equipment Co.). U. S. Patent 2,796,046. 3 claims. June 18, 1957. Offic. Gaz. U. S. Pat. Office, **719**, 3:496. 1957.

A drinking bowl into which water is admitted by a valve actuated by the nose of the animal. R. Whitaker

497. Antikicking device for cows. M. C. ANDERSON AND S. F. HANKS. U. S. Patent 2,796,849. 14 claims. June 25, 1957. Offic. Gaz. U. S. Pat. Office, **719**, 4:716. 1957.

A large clamp with shaped jaws conforming to the contour of the cow is placed over the back and waist of a cow. Gentle pressure is applied to the jaws by means of a screw, until the cow is restrained from kicking.
R. Whitaker

498. Comparative efficiency of some methods of estimating the live weight of dairy cows. A. W. BURT, N.I.R.D., Reading, England. J. Dairy Research, **24**: 144. 1957.

Data from 133 dairy Shorthorn cattle were used to relate various body measurements to weights of the animals. Regression equations relating the various body measurements to weights are given. Chest girth was the most satisfactory single body measurement by which to estimate weight. Chest girth, length of body, and paunch girth, used together along with age, gave an equation which yielded satisfactory estimates of weight. Other measurements explained little of the variance in estimated weights. The fiducial limits of estimated weights covered a range from four to five times as great as single actual weighings when chest girth alone was used, and this range was reduced by 30% when body length and paunch girth were included in the equation, as follows: Live weight (lb.) $= 0.000935\ \mathrm{I\,B(CG^2 + PG^2 + PG \times CG)} + 57$ (all measurements in inches). LB—Length of body: (withers to pins); CG—chest girth; PG—paunch girth. J. D. Donker

ICE CREAM

499. The worm turns in ice cream hardening. D. MERLIN. The Ice Cream Rev., **40**, 12: 26. 1957.

The new hardening room of the Southern Dairies plant at Norfolk, Virginia, is described. One of the unique features of the installation is a 1,000-foot overhead conveyor with a capacity of 8,000 gal. of ice cream. Packaged ice cream is loaded into baskets attached to the overhead conveyor by means of hooks. The ice cream is hardened as it is conveyed at near ceiling-height past a series of air blowers. The time required for a basket of ice cream to travel the length of the conveyor is nearly 5 hr. In actual practice, the elapsed time is considerably longer, due to intermittent operation of the conveyor. The hardened ice cream is removed from the baskets and stored in bins with no allowance for air circulation.

Advantages claimed for this method of conveying and hardening ice cream include increased capacity of the hardening room, reduction in number of men required in the hardening room, rehandling eliminated in the hardening room, and improved quality owing to faster hardening.

One disadvantage in the system cited concerns inventory control. It is suggested that this difficulty can be overcome by excluding from the inventory those items on the conveyor.

The construction of the hardening room differs from the conventional type in a number of respects. The floor is made of 16 in. of light insulating concrete. The walls consist of 8 in. of styrofoam with 1 in. of cement plaster on the inside and 5/16-in. of a cement coating on the outside. The ceiling has 8 in. of styrofoam with a built-up roof. The framework is steel. It is estimated that a saving of 20% is realized with this type of construction, as compared with the more conventional type construction.

W. J. Caulfield

500. Three-way savings. ANON. Ice Cream Rev., **40**, 12: 29. 1957.

The use of strong, light bags made of polyethylene for handling liquid ice cream mix at the Rochester Dairy Cooperative, Rochester, Minn., has resulted in reduced packaging, handling, and shipping costs. The bags have resulted in a saving of 0.6 cent per gal. of mix in packaging cost, as compared with the use of a conventional milk can. Truck-loading time has been reduced 57% by using the bags, and the payload per truck has been increased from 2,000 gal. of mix to 2,450 gal. by substitution of the polyethylene bags for conventional milk cans. Temperature rise of the product while en route has been only 3° F. over a 27-hr. period without refrigeration. W. J. Caulfield

501. Home-delivery formula. ANON., Ice Cream Field, **69**, 5: 36. 1957.

A brief account is given of the Kaydee Ice Cream Co., recently founded in Atlantic City, N. J., which operates exclusively in the home-delivery field. W. C. Cole

502. A study of stabilizers for new frozen desserts. H. J. BASSETT, E. F. Drew & Co., Inc., Boonton, N. J. Ice Cream Field, **70**, 1: 24. 1957.

The author discusses some of the functions of stabilizers and emulsifiers, and cites specific problems presented by ice cream manufacturers, which are solved by the proper use of such products. W. C. Cole

503. Seventy-three per cent of homes serve ice cream. ANON., Ice Cream Field, **69**, 5: 54. 1957.

A recent study entitled "Public Attitude and Uses of Dairy Products" has been completed for the American Dairy Association. It is concluded that about one-third of all homes have ice cream on an average day, and about one-half serve ice cream as dessert once a week. Ice cream is served as dinner dessert by 73% of all housewives. W. C. Cole

504. Dispensing scoop. A. GORDON (assignor to Ekco Products Co.). U. S. Patent 2,797,654. 2 claims. July 2, 1957. Offic. Gaz. U. S. Pat. Office, **720**, 1:78. 1957.

A hand-operated dipper for forming semi-spherical-shaped portions of ice cream or other foods. R. Whitaker

MILK AND CREAM

505. Kan oksydationssmak i mjölk motvirkes på biologisk måte ved hjelp av ekte mjölkesyrebakterier? (Can oxidized flavor in milk be prevented biologically by the use of homofermentative lactic acid bacteria?) P. SOLBERG AND G. HADLAND, Agri. Coll., Norway. Nord. Mejeri-Tidsskrift, **23**: 81. 1957.

Development of oxidized flavors in milk was prevented by addition of streptococcus lactic acid starters. Starters containing strongly reducing bacteria, as measured by the reduction of methylene blue and other dyes, gave the best results.

Two methods were employed. Milk was ripened with 1 to 2% starter at 15 to 28° C. for 30 to 135 min. prior to pasteurization, or 0.5 to 1% starter was added to milk following pasteurization. By either method, development of oxidized flavor was prevented during four days of storage.

The slight acid flavor developing in some samples by the second method was considered preferable to the oxidized flavor of the control samples. T. Kristoffersen

506. Looking at sanitary control. D. TIEDMAN, School of Public Health, Univ. of Michigan. Ice Cream Field, **69**, 5: 20. 1957.

The author takes a forward look at the official

control of sanitation of milk and milk products. He predicts that the next 50 years will bring less official dairy inspection on the farm, with a shifting of this responsibility to the industry. What must be accomplished from the standpoint of milk quality is considered of prime importance, as contrasted with emphasis on detailed inspection regulations.

In the author's opinion, there is no prospect that the treatment of milk by radiations, whether from high-speed electron accelerators or from radioisotopes, and whether of beta or gamma type, will replace pasteurization. He further states that properly pasteurized milk should not contain organisms of the coliform group. A new test for detecting coliform organisms is mentioned, as indicative of other improved laboratory tests which will be developed. W. C. Cole

MILK SECRETION

507. Mammary-gland development in the calf as a possible predictor of subsequent milk production. G. M. Elliott, Univ. of Reading, England. J. Dairy Research, **24**: 162. 1957.

First lactation records of 485 heifers were compared with their udder size at 4 mo. Included were 169 dairy Shorthorns in 17 herds, 75 Ayrshires in 7 herds, and 12 Friesians in 2 herds. The relationship between these two variables was negligible, and there was no basis for selection of milking animals on the basis of a 4-mo. udder palpation measurement.
J. D. Donker

508. Association between the fat percentage of cow's milk and the size and number of the fat globules. J. O. L. King, Univ. Liverpool, Vet. Field Sta., Neston, Wirral, England. J. Dairy Research, **24**: 198. 1957.

The milk of 56 Ayrshire and 40 Friesian cattle was examined for fat content, size of fat-globule, and number of globules per unit volume of milk between the 45th and 165th days after calving. In both breeds, the fat-globule size increased as the fat content of the milk increased, and in the Friesians the number of globules per cm. increased with fat-content increase. J. D. Donker

509. Relationship between milk secretion and the rate of milking by machine. P. A. Clough and F. H. Dodd, N.I.R.D., Reading, England. J. Dairy Research, **24**: 152. 1957.

The records of 352 first-calf heifers of Dairy Shorthorn, Friesian, and the Ayrshire breeds were analyzed to relate yield of milk in a lactation to the maximum rate of milk flow achieved by machine-milking under standard conditions. The animals were divided into seven groups, according to year and breed, for statistical analysis. The regressions of yields upon peak flows were positive in all groups, but only significantly so ($P > 0.05$) in four out of seven cases. The highest correlation between the two variables studied was 0.534. Regression and correlation coefficients for the total group were highly significant at the 1.0% level of probability. The authors stated that selection of dairy animals on the basis of milking rates, as a means for increasing yields, would prove unfruitful.
J. D. Donker

510. Intramammary pressure changes in the lactating ewe. I. The nature of the pressure change under standard conditions. W. G. Whittlestone. Ruakura Animal Research Sta., Dept. of Agr., N. Z. J. Dairy Research, **24**: 165. 1957.

Three ewes were used in a series of oxytocin-injection experiments, to determine the characteristics of the intramammary pressure changes associated with milk ejection. The pressure pattern developed was compared with that of the pig and cow, and was intermediate in the matter of fall of pressure, once a maximum had been reached. The pressure in the sow recedes very rapidly, and that of the cow is maintained for a long period.

An improved device for measuring and recording intramammary pressure was described.
J. D. Donker

PHYSIOLOGY AND ENDOCRINOLOGY

511. Influence of some factors on the relative evaporation rate from the skin of cattle. A. Buman. Nature, **179**, 4572: 1256. 1957.

Moisture evaporation was measured by noting the time required to cause a color change in a strip of filter paper, previously soaked in 10% cobalt chloride and thoroughly dried. The paper was observed in a small glass cup, pressed against the skin. The following factors influenced the rate of evaporation: location on body, the greatest being in areas having the greatest sweat-gland distribution; color of skin, greater evaporation from darker-colored areas; shearing, shearing increases rate; and individual cow characteristic, a property apparently inherited from the sire. R. Whitaker

512. Reviews of the progress of dairy science. Section A. Physiology of dairy cattle. Hormones in reproduction and lactation. G. K. Benson and A. T. Cowie. N.I.R.D., Reading, England. J. Dairy Research, **24**: 252. 1957.

This review covers much of the material from 1953 to date in the field of its topic. A total of 392 publications has been reviewed and categorized into the following main classifications: Hormones in Body Fluids of Ruminants, Hormone Content of the Pituitary Gland, Hormonal Control of the Estrous Cycle, Hormones and Infertility, Pineal Gland and Mammary Growth and Lactation, each usually including several subheadings. J. D. Donker

513. Effect of thyrotropin on milk yield and heart rate of the goat. E. C. Owen and R. A. Darroch, The Hannah Dairy Research Inst., Kirkhill, Ayr., Scotland. J. Dairy Research, **24**: 157. 1957.

Five British Saanen goats were utilized to determine the galactopoietic and heart-rate stimulating effects of growth hormone and thyrotropin. During a first period, all were treated alike and, during a second period, two received growth hormone and two thyrotropin, and the fifth remained as control. During a third period, all remained untreated.

Growth hormone caused increased milk production and rate of heart-beat. Thyrotropin caused a large increase in milk production in one case, but not in the other, and increased heart-rate in both. J. D. Donker

SANITATION AND CLEANSING

514. Sanitary pipeline milking systems. H. P. Stephan, Universal Milking Mach. Co., Waukesha, Wis. S. Dairy Prod. J., **60**, 6: 56. 1956.

The use of pipelines in milking saves time and labor, and helps to eliminate contamination of the milk. Sanitary milk pipelines have no advantage for the farmer unless they are cleaned in place. Recommendations of the Sanitary Standards Committee for C.I.P. Milk Pipelines on Dairy Farms should be followed, for satisfactory installation and cleaning. All ells and risers should be eliminated if possible, the operation of partially filled pumps avoided, and the line sloped 1 in. in 10 ft. toward the milkhouse. Fly and dust protectors should be provided for all milk-line openings, and automatic traps installed on vacuum lines to drain out moisture. Approved detergents are important. The solutions may be recirculated by a pump or by vacuum. The recommended time, temperatures, velocity, and concentration of detergent solutions for any effective C.I.P. system should apply. F. W. Bennett

515. Spray systems. A new method of cleaning pans, tanks, and enclosed equipment. Part One. R. D. Bovey, Klenzade Products, Inc., Salt Lake City, Utah. S. Dairy Prod. J., **60**, 4: 246. 1956.

The cleaning of vacuum pans, hot wells, milk-storage tanks, and truck tanks allows the use of stronger detergent solutions and higher temperatures than in the more conventional and manual methods. Spray arms and nozzles, either stationary or rotating, should be designed for applying detergent solutions to each type of equipment. Heat generally is applied to the solutions by steam injectors. A typical procedure for cleaning a vacuum pan by the spray method includes rinsing with tepid water, circulating 2 to 4% caustic solutions at from 190 to 200° F. for from 45 to 60 min., allowing the solution to stand and cool to 130° F. and draining, circulating an organic acid detergent solution at pH of 4.0 and temperature of from 135 to 140° F. for from 20 to 30 min., draining, rinsing with water not over 100° F., opening, and hand-brushing where needed. Chelation of the caustic solutions may be advisable. Systems should be free of dissimilar metals, to avoid electrolysis. The spray system of cleaning makes possible savings in labor, water, steam, and detergents, and produces more satisfactory results. F. W. Bennett

516. Spray systems. A new method of cleaning pans, tanks, and enclosed equipment. Part Two. C. Orr, Klenzade Products, Inc., Beloit, Wis. S. Dairy Prod. J., **60**, 4: 248. 1956.

Certain precautionary measures must be taken to insure a proper spray-cleaning job, and to remove the possibility of injury of the operator or damage to the equipment. All lines in the system should be set up with approved C.I.P. fittings, nonleaking ground joints, rubber gaskets, or welded joints. Pressures in the equipment must be avoided by leaving an opening, preferably vented to the outside of the building. A thermometer and pressure gauge should be installed in the line of flow, and pressure from more than one pump should be equalized. Pumps, pipelines, fittings, and nozzles should be constructed of resistant stainless steel. F. W. Bennett

517. Dairy waste prevention and treatment. The Subcommittee on Dairy Waste, Dairy Industry Committee, Washington. S. Dairy Prod. J., **60**, 4: 216. 1956.

Ways and means for preventing dairy waste and for treating dairy wastes at low cost have been studied for several years. The recommended waste prevention program involves the active participation of company executives, education of plant personnel, and proper selection, operation, and maintenance of equipment. Brief progress reports are made on research dealing with treatment of dairy wastes, at Michigan State Univ., Purdue Univ., Eastern Utilization Research Branch of the USDA, Pennsylvania State Univ., Iowa State College, and the Univ. of Wisconsin. The best of several satisfactory systems for an individual situation should be selected in consultation with competent dairy sanitation-engineers. F. W. Bennett

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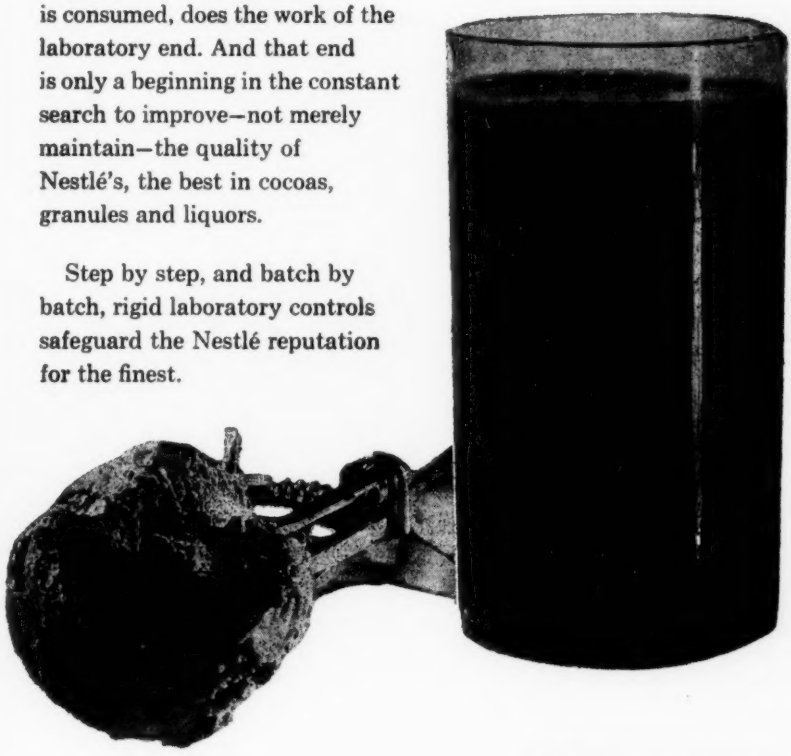

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